

SEA WATER AS A MEDIUM FOR TISSUE CULTURES

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FOUR FIGURES

As early as 1878 L. Fredericq demonstrated that the blood and haemolymph of invertebrate marine forms is isotonic with the sea water and it has long been known that Locke's solution and Ringer's solution contain the same proportion of certain salts as does the sea water, only in different concentrations (Loeb, '06). Macallum ('08) has shown that not only does the body fluid of the lower forms of marine life correspond in composition with that of sea water, but also that the plasma of higher animals is not changed in composition from sea water, but is simply more dilute.

Loeb ('06) in his work on the antagonistic action of certain salts, demonstrated the necessity for the presence of the various salts in the composition of sea water, also the importance of the proportion of the various salts one to another in order to obtain normal behavior of certain marine animals.

Although the composition of the sea water varies slightly for different localities, on the whole the salt content remains surprisingly constant, and while an analysis of the sea water for practically any given locality has been determined, it is in general that given by Henze ('10) (taken from Roth):

Ocean surface

To 1000 parts

NaCl	26.862	
KCl	0.582	
MgCl ₂	3.239	
MgSO ₄	2.196	Cl. 18.999
CaSO ₄	1.350	
Rest	0.070	
	34.299	

Middle ocean

<i>To 1000 parts</i>		
NaCl	30.292	
KCl	0.779	
MgCl ₂	3.240	
MgSO ₄	2.638	Cl. 19.999
CaSO	1.605	
Rest	0.080	
38.634		

Herbst, C., '03-'04, makes artificial sea water as follows:

<i>To 100 cc. of distilled water</i>	
NaCl	3.00 g.
KCl	0.08 g.
MgSO ₄	0.66 g.
CaCl ₂	0.13 g.

To 100 cc. of above he adds 1 cc. of a 4.948 per cent solution of NaHCO₃. Loeb, J., '13, uses 100 molecules NaCl, 2.2 molecules KCl, 7.8 molecules MgCl₂, 3.8 molecules MgSO₄ and to this he adds 1-2 molecules CaCl₂. This solution is diluted until specific gravity corresponds with that of the sea water normal for the animal and for regeneration problems Loeb adds to 100 cc. of the above solution 1 cc. of a 3/8 m. NaHCO₃ solution.

While the ratios of certain salts are quite constant, there are other variations which influence the osmotic pressure of the sea water in different localities, as for instance Loeb, J., '13, states that the free alkalinity is higher in the sea water at Woods Hole than at Pacific Grove.

The osmotic pressure of a solution such as the sea water or the plasma can be determined according to Garrey ('15) from the depression of the freezing point of the solution by means of the formula : osmotic pressure = $22.4 \Delta / 1.85$.

Garrey ('15) states that the determinations of the sea water at Woods Hole for six different years gave an average $\Delta = 1.81^\circ\text{C}$., and that this is isotonic with sodium chloride 0.52 m; magnesium chloride 0.29 m; cane sugar 0.73 m and Van't Hoff's

TABLE 1—GARREY

SEA WATER FROM	Δ —°c	OBSERVER	REFERENCE
Naples.....	— .229	Bottazzi	Arch. ital. de biol. 1897 v. 28, p. 61.
Arcachon.....	— 1.89	Rodier	Trav. des Lab. d'arcachon, 1899, 103.
Pacific Grove, Cal.....	— 1.925	Greene	Bull. U. S. Bureau Fisheries, 1904, v. 24, p. 429.
Pacific Grove, Cal.....	— 1.90	Garrey	Biol. Bull., 1905, v. 8, p. 257.
Woods Hole.....	— 1.81	Garrey	Biol. Bull., 1905, v. 8, p. 257.
Beaufort, N. C.....	— 2.04	Garrey	1911.
Helgoland.....	— 1.90	Dakin	Bio.-Chem. Jour., 1908, 269.
In the Kattegat.....	— 1.66	Garrey	
Open Baltic Sea.....	— 1.30	Dakin	
Kiel Harbor.....	— 1.093	Dakin	
Newport River near Beaufort.....	— 1.707	Garrey	Biol. Bulletin, vol. 28, no. 2, 1915.

solution made up from half molecular solutions according to the formula given by Loeb '13 (see above).

Garrey also states that by his findings the sea water of Pacific Grove is about 5 per cent more concentrated than that of Woods Hole, while Beaufort, N. C., is about 12 per cent more concentrated than Woods Hole.

The osmotic pressure of the plasma of many animals can be determined from the Δ (depression of freezing point of their plasma) given below.

In addition to the above Garrey '15 has determined the freezing point for various dilutions of sea water and also the concentration of pure salt which has a corresponding freezing point.

Thus it can be seen that if the Δ for the plasma of any given animal is known, it is an easy matter with the aid of the above table to find the dilution of sea water which is isotonic with the plasma in question and thus one can obtain a solution, which contains the salts in the same proportion as the plasma and at the same time isotonic with the plasma.

This general formula does not hold for the selachians, for the blood of these animals contains a large amount of urea and

TABLE 2

ANIMAL	$\Delta - ^\circ\text{C.}$	LOCALITY	OBSERVER
Invertebrate Marine Animal.			
Aleyonium palmatum.....	-2.196		Bottazzi
Asteropecten aurantiacus.....	-2.312		Bottazzi
Holothuria tubulosa.....	-2.315		Bottazzi
Sipunculus nudus.....	-2.31		Bottazzi
Maja squinado.....	-2.36		Bottazzi
Homarus vulgaris.....	-2.29		Bottazzi
Octopus macropus.....	-2.24		Bottazzi
Limulus polyphemus.....	-1.82	Woods Hole	Garrey
Limulus polyphemus.....	-2.03	Beaufort	Garrey
Limulus polyphemus.....	-1.71	Newport river near Beaufort	Garrey
Vertebrate Marine Animal.			
Elasmobranches.....	-2.182	Beaufort	Garrey
Torpedo marmorata.....	-2.26		Höber
Mustelus vulgaris.....	-2.36		Höber
Trygon violacea.....	-2.44		Höber
Charax puntazzo.....	-1.04		Höber
Cerna gigas.....	-1.035		Höber
Crenilabrus pavo.....	-0.74 -0.76		Höber
Box salpa.....	-0.82 -0.88		Höber
Chelonia mydas.....	-0.675	Beaufort	Garrey
Colpochelys kempi.....	-0.687-0.7	Beaufort	Garrey
Caretta caretta.....	-0.69 -0.625	Beaufort	Garrey
Thalassochelys caretta.....	-0.61		Bottazzi
Chelonia caonana.....	-0.60		
Cetacians.....	-0.65 -0.7		
Pinnipeds.....	-0.65 -0.7		
Invertebrate fresh water anima.			
Anodonta cygnea.....	-0.20		Höber
Astacus fluviatilis.....	-0.80		Höber
Dytiscus marginalis.....	-0.57		Höber
Hirudo medicinalis.....	-0.40 -0.43		Höber
Libellen larva.....	-0.6		Höber
Libellen Imagin.....	-0.65		Höber
Vertebrate fresh water animals			
Anguilla vulgaris.....	-0.58 -0.69		Höber
Barbus fluviatilis.....	-0.475-0.558		Höber
Leuciscus dobula.....	-0.45		Höber
Cyprinus carpio.....	-0.512		Höber
Tinea vulgaris. Esox lucius.....	-0.512		Höber
Salmo trutta.....	-0.62		Höber
Polyodon spathula.....	-0.486-0.50	Mississippi river	Garrey
Scaphirhynchus platyrhynchus.....	-0.503-0.507	Mississippi river	Garrey
Lepidosteus osseus (L.) ('bar').....	-0.487-0.52	Mississippi river	Garrey
Amia calva (L.) (landlocked).....	-0.508	Mississippi river	Garrey
Catostomus teres.....	-0.51 -0.52	Mississippi river	Garrey
Perca fluviatilis.....	-0.498-0.51	Mississippi river	Garrey
Fresh water ganoids all have blood identical in concentration with fresh water teleosts.			
Rana esculenta.....	-0.465		Bottazzi
Salamandra maculata.....	-0.479		Bottazzi
Emys europaea.....	-0.474		Bottazzi
Pseudemys elegans.....	-0.48	Mississippi valley	Garrey
Man.....	-0.526		Hamburger
Cow.....	-0.585		
Horse.....	-0.564		
Pig.....	-0.615		
Rabbit.....	-0.592		
Dog.....	-0.571		
Cat.....	-0.638		
Sheep.....	-0.619		

TABLE 2—Continued

EGGS AND EMBRYOS	Δ	OBSERVER
<i>Frog</i>		
Blood of frog.....	-0.46	Backman and Runnström
Ovarian egg.....	-0.46	Backman and Runnström
Fertilized unsegmented egg.....	-0.045	Backman and Runnström
Early gastrula.....	-0.42	Backman and Runnström
Late gastrula.....	-0.215	Backman and Runnström
Early medullary plate.....	-0.215	Backman and Runnström
5 day embryo.....	-0.230	Backman and Runnström
20-25 day embryo.....	-0.405	Backman and Runnström
<i>Chick</i>		
Blood of chick.....	-0.63	Bialaszewicz
Ovarian egg.....	-0.63	Bialaszewicz
Egg yolk.....	-0.45	Bialaszewicz
Embryo incubated 6 days.....	-0.508	Bialaszewicz
8 days.....	-0.517	Bialaszewicz
10 days.....	-0.523	Bialaszewicz
12 days.....	-0.557	Bialaszewicz
14 days.....	-0.560	Bialaszewicz
16 days.....	-0.566	Bialaszewicz
18 days.....	-0.601	Bialaszewicz

The records of Bottazzi are quoted from Höber ('06).

probably the culture medium most favorable for these animals would be one made up according to Fühner, H. ('08).

To 1000 parts distilled H_2O .

Sodium bicarbonate 0.2 gr.

Calcium chloride dry 0.2 gr.

Potassium chloride 0.1 gr.

Sodium chloride 20.0

Urea 25.0

or else one made up from $66\frac{2}{3}$ sea water + $33\frac{1}{3}$ distilled H_2O (which has the same Δ as a 2 per cent NaCl solution) with sodium bicarbonate 0.02 g. and urea 2.5 g.

Also A. R. Koontz, who is now working on the fresh water muscles finds that manganese is probably necessary for certain fresh water muscles.

By actual experiment, as can be seen below, a solution which is slightly hypotonic to the blood plasma forms a more successful tissue culture medium than one which is exactly isotonic.

TABLE 3—GARREY

DILUTION: WOODS HOLE } + { DISTILLED SEA WATER } WATER C.C.M. C.C.M.		$\Delta = ^\circ\text{C.}$	DENSITIES OF SEA WATER DILUTIONS AT 21.5°C. (REF. H_2O AT 21.5°C.)	NaCl WITH SAME GMS. IN 100 CC. OF SOLUTION
cc.	cc.			
Undiluted	0	-1.81	1.02426	3.04
85	15	-1.54		2.6
75	25	-1.35		2.275
66 $\frac{2}{3}$	33 $\frac{1}{3}$	-1.20		2.00
60	40	-1.09		1.81
50	50	-0.915	1.0123	1.58
45	55	-0.82		1.4
40	60	-0.73	1.0096	1.21
35	65	-0.64		1.07
33 $\frac{1}{3}$	66 $\frac{2}{3}$	-0.61	1.008	1.02
32	68	-0.595		1.00
30	70	-0.547	1.0073	0.91
25	75	-0.460	1.0062	0.76
20	80	-0.37	1.0046	0.60
10	90	-0.187	1.0023	0.30

Loomis '94 (quoted from Höber).

Mol. per liter	Percentage of NaCl	Δ
0.01	0.058	-0.036
0.02	0.117	-0.071
0.03	0.176	-0.106
0.04	0.234	-0.141
0.05	0.293	-0.176
0.06	0.351	-0.211
0.07	0.410	-0.245
0.08	0.468	-0.280
0.09	0.527	-0.314
0.10	0.585	-0.348
0.20	1.17	-0.687

This is not actually true, for when all operations are carried on in a moist chamber, the isotonic solution is satisfactory, but since tissue cultures are usually made in the dry air of the laboratory where extensive evaporation takes place, the hypotonic solution gives more successful results. In fact a solution which is quite hypotonic may give good growth for the cells appear to absorb large quantities of water without injury, but a hypertonic solution retards the growth of the cells.

For animals the Δ of whose plasma has not been determined, the sodium chloride content of the plasma may be used by means of table 3-Garrey to find the dilution of sea water which is isotonic with that percentage of sodium chloride. The sodium chloride content of the plasma of many animals may be found by means of the table of Fürth ('02), who gives an analysis of the plasma of many lower animals.

As Loeb, J., ('06) has suggested, the above solutions are in reality more protective than nutritive in their action and it is necessary to add some substance for the nourishment of the tissue, provided the culture is to be kept under observation for several days. Dextrose 0.1 per cent, 0.25 per cent and 0.5 per cent and a bouillon containing peptone¹ serves for this nutritive substance.

One other point must be kept in mind in regard to a medium for tissue cultures and that is the fact that acid, even a slight trace of acid, interferes with growth and in order to keep the culture medium alkaline it is necessary to add about 0.02 per cent sodium bicarbonate.

The temperature at which the tissue cultures should be kept is that indicated by the body temperature of the animal in question, i.e., warm blooded animals at 39° C. and cold blooded at more or less the temperature of the normal environment of the animal. Although the temperature of the ocean is quite constant, necessarily that of the aquarium and also of tissue culture varies decidedly according to the room temperature and, while no tests were made, certain of the tissue cultures of cold blooded animals grew well at a room temperature which must certainly have been much higher than that normal for the environment of the animal.

¹ 500 grams (one pound) finely chopped muscle is placed in a liter of distilled water and kept on ice for twenty-four hours. It is then cooked, strained and filtered and sufficient distilled water added to bring the fluid up to a liter again. 10 grams of peptone, pure (Witte's) 5 grams of NaCl are added and the medium heated to dissolve the peptone. It is then carefully neutralized, filtered and sterilized. This bouillon can be kept for days. In place of bouillon, 0.1 per cent or 0.2 per cent peptone alone may be used with satisfactory results.

SUMMARY

From the above it can be seen that a medium for tissue cultures can be formed as follows: 90 cc. of the dilution of sea water (or of Locke's solution), which is isotonic with the plasma of the animal from which the cultures are to be made, + 10 cc. of bouillon made from the muscle of the animal in question. It has been found that about 10 cc. of the bouillon, in a few cases 15 cc. or even 20 cc. were necessary, make the solution sufficiently hypotonic to offset any evaporation which may take place and at the same time to furnish the necessary nitrogenous food for the tissue. To the above 90 cc. of diluted sea water + 10 cc. of bouillon is added 0.02 grams of NaHCO_3 to neutralize the acid formed by the culture and 0.25 grams of dextrose to supply the energy for growth of the tissue. The cultures must be aseptic and should be kept in a warm chamber at 39°C . when made from the tissue of a warm blooded animal, or at the temperature normal for the animal when the cultures are made from tissue of a cold blooded animal.

EXPERIMENTS

Chick. In the case of the chick embryo (5 to 9 days) the sea water was made up from dry sea salt and possibly thus contaminated with various foreign substances. However that may be, the medium thus obtained gave successful growth, although on the whole the growth was neither as large nor did it contain as many mitotic figures as the growth obtained from a piece of chick limb bud when explanted into Locke's solution. Pieces of chick embryo heart explanted into the sea water medium did not survive as well as those in Locke's solution. The media most favorable for growth were 1) 30 cc. sea water + 60 cc. distilled water + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose + 10 cc. chicken bouillon, and 2) 90 cc. distilled water + 10 cc. chicken bouillon + 0.9 per cent sea salt + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose.

The growth from explanted pieces of the embryo limb bud is composed of abundant connective tissue and some muscle fibres

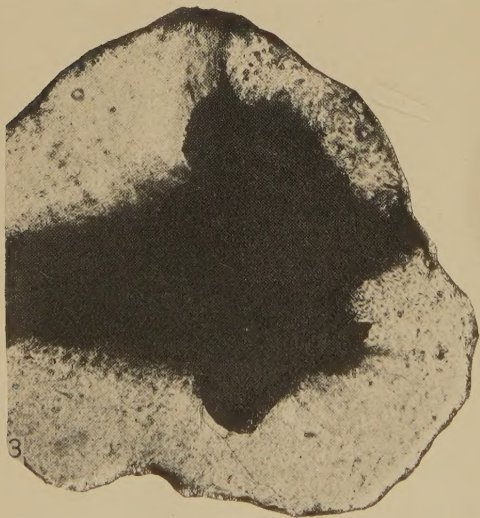
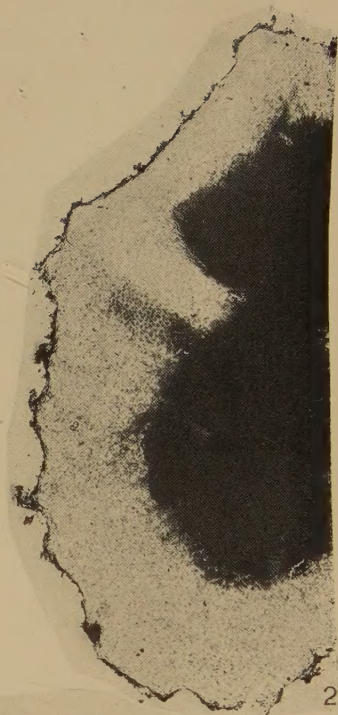
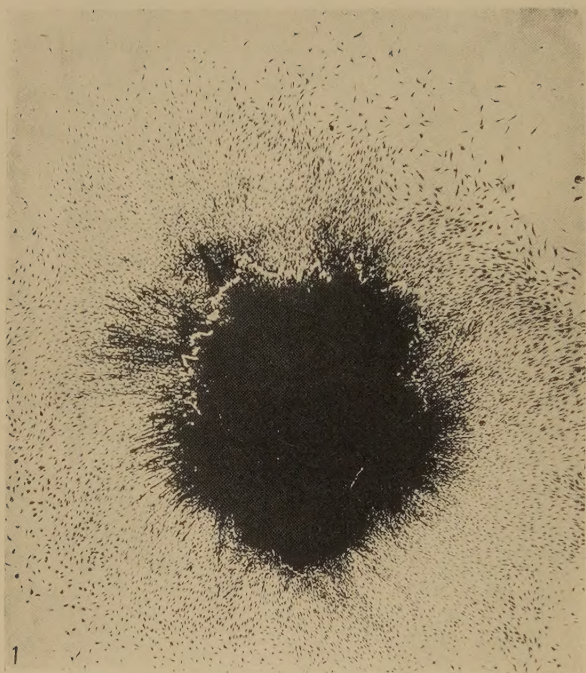
(fig. 1), and in some cases a patch of epithelial membrane grows out in the midst of the connective tissue cells. The growth in sea water continued active for from 4 to 6 days and many growths were kept in a healthy condition for as long as 14 to 20 days by washing the culture every other day with a fresh drop of the same culture medium.

Contraction of the muscle fibres was not observed as was observed when the fibers grew out from a piece of chick limb bud and explanted into Locke's solution (Lewis, M. R., '15).

Fundulus. The media were made up from fresh sea water and the dilution which gave the largest growth was 40 cc. sea water to 60 cc. of distilled water. This is much more dilute than that given by Garrey (45 cc. sea water to 55 cc. distilled water) for the fundulus, but the above gave more successful results than did that of Garrey.

A fundulus egg, which contained an embryo just about to hatch, was placed in several successive dishes of sterile sea water by means of a sterile pipette and then from the sterile sea water into a dish of sterile medium 80 cc. of diluted sea water (40 cc. sea water + 60 cc. distilled water) + 20 cc. fundulus bouillon + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose. In this dish the egg membrane was torn away as rapidly as possible and the embryo at once transferred to another dish of sterile medium, in which the embryo was cut into about six small pieces and each piece again transferred to a dish of sterile medium from which the hanging drop cultures were made in the usual manner (Lewis and Lewis, '15).

The cut surface of many of the explanted pieces simply became covered over by a growth of epithelial cells, but the pieces from the body of the embryo, especially those from the region where the yolk sac was attached, gave rise to the typical tissue culture growth along the cover slip. This growth spread out from the body wall as a thick, firm membrane. The epithelial cells, easily distinguished by their characteristic markings, formed the lower layer of this membrane, and over this grew numerous other cells, some of which were connective tissue cells and some appeared to be blood cells. The growth remained active for



from 3 to 6 days. The muscle fibers continued to twitch spasmodically, but did not grow out as they did in the case of the chick. Few mitotic figures were seen. No effort was made to prolong the life of the cultures by washing it with fresh medium.

Hermit crab. The medium most favorable for growth was as follows: 90 cc. sea water + 10 cc. crab bouillon which contained 1 per cent NaCl + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose. Very few of the tissues of the hermit crab grew as tissue cultures. The best results were those from the first claw which was undergoing regeneration, although the membrane which forms the breaking joint and also the lining of the shell gave rise to good growth when explanted.

Figure 4 shows the type of growth which arises from an explanted piece of the membrane of the breaking joint or from the lining of the shell. These cells are much smaller than those of either the fundulus or of the chick growths. They never form a membrane. Pieces of the regenerating first claw explanted into the above medium gave rise to extensive growth composed of a small cell either connective tissue or epithelium and a few muscle fibers. Unfortunately no good fixed preparations of these growths were obtained for photographs.

Limulus. The explanted pieces of shell lining gave about the same results as did those of the crab and the medium used was the same as that for the crab. The heart and skeletal muscle

Fig. 1 Photograph of a 4-day growth from the limb bud of a 6-day chick embryo explanted in sea salt 0.9 per cent + NaHCO_3 0.02 per cent + dextrose 0.25 per cent + 90 cc. distilled H_2O + 10 cc. chicken bouillon. Connective tissue and radiating muscle fibres. Osmic acid vapor fixation and iron haematoxylin stain. $\times 38$.

Fig. 2 Photograph of a 3-day growth from a piece of fundulus embryo explanted into 80 cc. of diluted sea water (40 cc. sea water + 60 cc. distilled H_2O) + 20 cc. fundulus bouillon + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose. The membrane is not contracted. Osmic acid fixation and iron haematoxylin stain. $\times 38$.

Fig. 3 Same as figure 2 only the edge of this membrane is contracted.

Fig. 4 Photograph of the growth from a piece of hermit crab breaking joint membrane explanted into 90 cc. sea water + 10 cc. crab bouillon which contained 1 per cent NaCl + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose. Osmic acid fixation and iron haematoxylin stain. $\times 50$.

gave rise to only a few wandering cells and the pericardium gave rise to many active wandering cells, the exact nature of which was not determined.

Sea anemone. The medium used was as follows: 95 cc. sea water + 5 cc. distilled water + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose + 0.1 per cent peptone. Pieces of the septa explanted into this medium gave rise to an abundant growth, which was composed of a substance probably mesogloea and numerous scattered endoderm cells. The cilia along the edge of the septa remained active for many days in the cultures.

Grasshopper. As there was no analysis of the grasshopper's plasma found, the most favorable dilution of the sea water for growth of the grasshopper tissue was determined by a series of trials of various dilutions. 30 cc. sea water + 50 cc. distilled water + 20 cc. grasshopper bouillon + 0.02 per cent NaHCO_3 + 0.25 per cent dextrose was the medium which gave the best results.

The only tissue studied carefully was that of the testis, and it was found that not only the cell within the follicles, but also the isolated sperm cells, remained in a healthy condition and continued to divide in this medium. The behavior of the cytoplasmic structures of the sperm cells were studied carefully and will be given in detail in a separate publication.

The results from the cultures made do not justify any conclusions as to the comparative value of any one medium for tissue cultures, but they do show that the dilution of sea water affords a simple and exact way by means of which can be obtained a medium, which is not only isotonic with the plasma of any given animal, but which also contains the necessary salts in the same proportion as does the plasma of the animal.

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EXPERIMENTAL MESOTHELIUM

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ELEVEN FIGURES

In a normal animal that survives, repair and sometimes regeneration follows the destruction of tissue. The phenomenon of regeneration, that results in the restoration of structure and function has long been studied and discussed.

Following a physical injury which destroys the free surface cells of the peritoneum or pleura, or the lining cells of blood vessels, two possibilities exist as to how regeneration of the damaged zone proceeds. 1) Cells may grow from the periphery of the given denuded area, taking origin from adjacent, previously existing and intact flat surface cells; or 2) the exposed connective tissue cells of the floor of the injured area may proliferate, be changed in form and become flattened. In the second possibility, the pressure of the opposed surface of the smooth normal peritoneum covering either intestine or other organ is the physical agent, acting continually, that is premised as tending to flatten the surface cells of the damaged area. The pressure and friction of the rushing stream of blood is premised as acting in a similar manner upon the exposed proliferating cells following the destruction of the lining endothelium of a vessel. If there exists a parietal coagulum, reparative cells may invade it and become flattened by the blood stream when the surface of the coagulum is reached. If no coagulum is attached to the denuded area the proliferating cells are immediately exposed to the pressure and friction of the stream of rushing blood. Active friction and pressure between surfaces physically dissimilar, will always result in a mutual change in form until a physical equilibrium is established between them.

The experiments were undertaken in reference to the second possibility; namely, that the exposed deep connective tissue cells making up the floor of the injured area proliferate, change in form and become flattened; thus resulting in the so-called regeneration of a lining membrane in the repair of the surface cells. In other words, an attempt was made to ascertain what change in form takes place in the investing living connective tissue cells in contact for a time with the surface of various, relatively non-irritating, foreign bodies.

EXPERIMENTS

Celloidin is readily moulded into any shape, and after drying may be sterilized by boiling. At the same time celloidin, after the alcohol and ether have evaporated, sets free no chemicals in the dry state when placed in the tissues. Since 1910 more than sixty experiments have been done in which celloidin has been placed in the tissues of several varieties of animals and allowed to remain even as long as one year before removal for study. Its use has always resulted in the formation of a minimum amount of adventitious tissue about the foreign body. After the cellular gathering had disappeared, that is found in and about tissue debris immediately after the operative procedure, neither leucocytes nor so-called 'giant cells' were found in proximity to the celloidin. Celloidin was therefore accepted as relatively ideal non-irritating foreign body for use in operative procedures and experimentations in living tissues.

Experiment No. 1. Celloidin foreign body. Duration 28 days. Dog. Animal No. 33. Operation October 3, 1913. Through a small wound of the skin of the abdominal wall, the subcutaneous tissues were divulsed by thrusting into the areolar tissue a closed artery forceps, opening the instrument, and withdrawing it while forcibly held open. This injury produced a long, relatively dry tract. Following the introduction of a thin, sterile, smooth sheet of celloidin $2'' \times \frac{1}{2}'' \times \frac{1}{6}''$, the tissues immediately collapsed permitting an easy closure of the skin wound. The wound healed by primary union and the thin strip of celloidin could be felt beneath the skin.

The foreign body together with the adventitious tissue, was removed October 31, 1913, and the specimen (Surg. Path. No. 2642) was fixed in formalin. Paraffin embedding. Sections were cut at

right angles to the free surface. The surface cells, as the section shows, were elongated, closely disposed and parallel to the free surface. Tangential sections were also cut to demonstrate the free surface cells or those in contact with the foreign body, the celloidin strip. The cells were close together and were proven to be flat since the two planes in which the sections were cut were at right angles to each other. Compare figures 2 and 3 with figure 1.

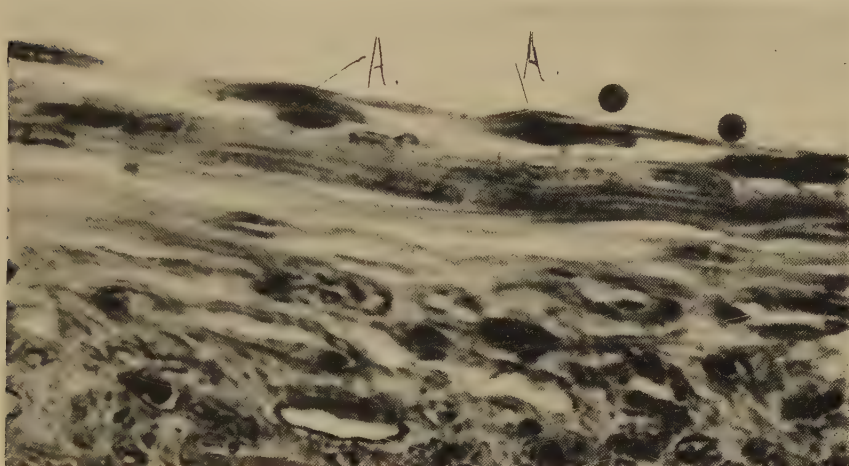


Fig. 1 Photomicrograph, mag. 900. Experiment 28 days. Cross section, showing surface cells in contact with a solid, smooth, flat, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642. See A. A.

Since a histological criterion of mesothelium is a mosaic of black silvered lines, which results when the fresh peritoneum and certain other free surfaces are treated with silver salts, this test was made in the next experiment.

Experiment No. 2. Celloidin foreign body. Duration 21 days. Dog. No. 104. Operation December 5, 1913; a repetition of Experiment No. 1, up to the point of excision of foreign body and adventitious tissue which was done on December 26, 1913. The tissue (Surg. Path. No. 2744) enclosing the celloidin was split open, particular care being taken not to physically injure the specimen. The tissue in contact with the smooth surface of the celloidin was smooth and glistening. A square of the lining surface was placed for one hour in a protargol solution of about 20 per cent. The specimen was then placed in 40 per cent alcohol and exposed to the sunlight. As soon as the

tissue had become a deep brown, frozen sections were cut tangential to the free surface that had been in contact with the celloidin plate. The mosaic of silvered lines in this free surface is shown in figure 4.

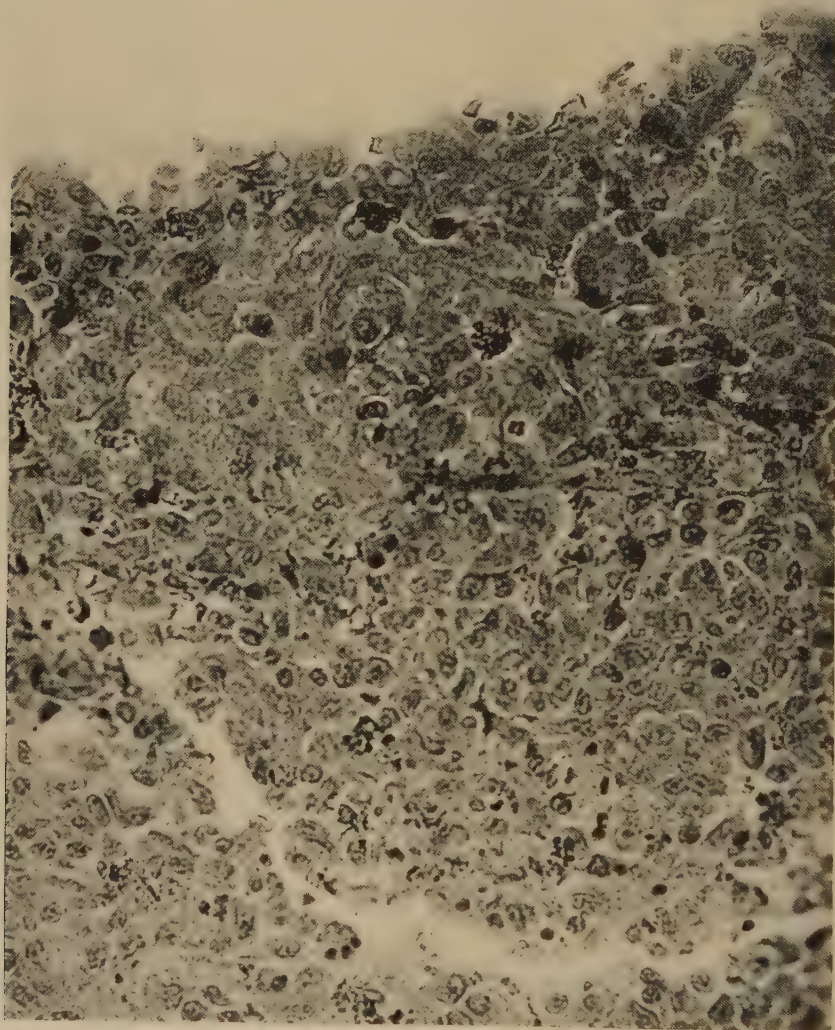


Fig. 2 Mag. 450. Experiment 28 days. Tangential section showing surface cells on the flat in contact with a solid, smooth, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642.

This silver mosaic demonstrated conclusively that the surface is covered by a complete layer of cells, and that the intercellular substance, as far as the silver reaction is concerned, is similar chemically to the

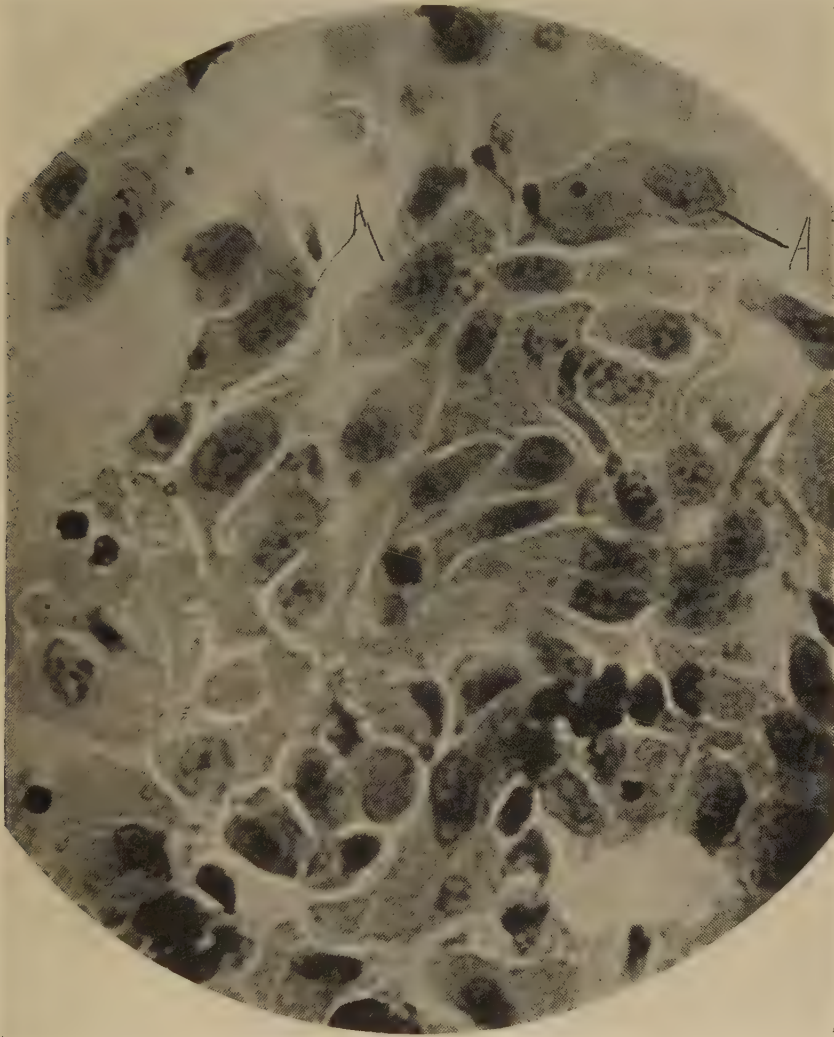


Fig. 3 Mag. 1000. Experiment 28 days. Tangential section showing surface cells on the flat in contact with a solid, smooth, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642.

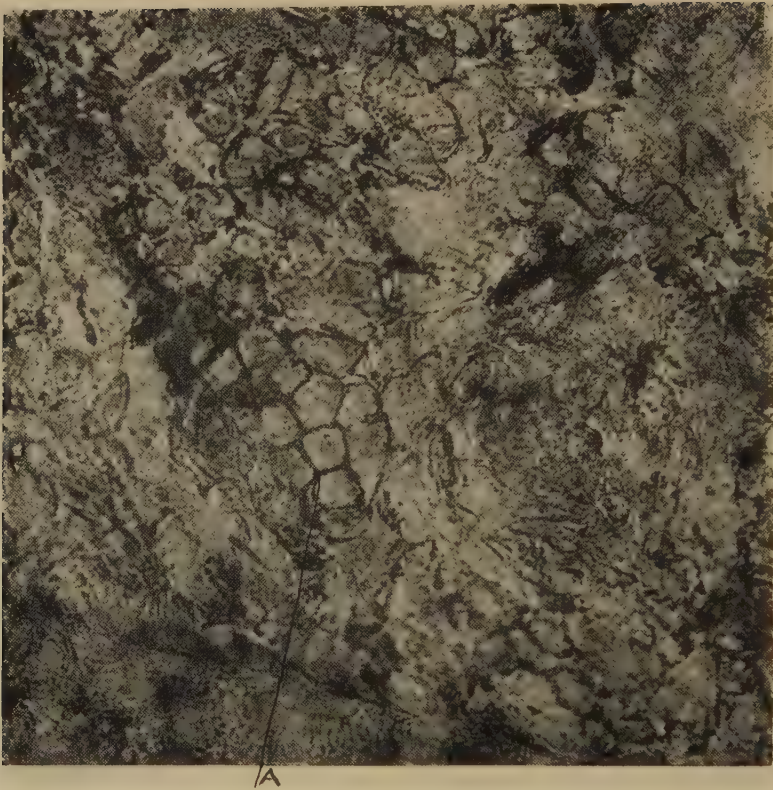


Fig. 4 Mag. 500. Microphotograph experiment. Silver mosaic in the free surface of tissue in contact with the smooth surface of celloidin. Specimen from the subcutaneous tissue of dog No. 2744. See A.

intercellular substance of the peritoneum, pleura and endothelium of blood vessels.

Paraffin has been used for years to fill cavities in the tissues, and this material forms a solid foreign body, comparatively non-irritating.

Experiment No. 3. Paraffin foreign body. Duration 19 days. Rabbit. Operation December 18, 1911. Melted paraffin was injected through a fine needle into the substance of the cornea. This injection mass spread out in a triangle with the apex at the sclero-corneal junction, the point where the needle had been introduced, and extended outwards over the pupil. The paraffin presented a feathery

appearance in the transparent cornea. The paraffin had been split up into small isolated columns and rod-like shapes when shot into the tissues. These shapes had been preserved when the paraffin solidified. For four days new blood vessels running in from the sclero-corneal junction were seen on the outer margin of the paraffin mass. All these vessels diminished continuously and had disappeared when the cornea was removed, January 6, 1912 (Specimen Surg. Path. No. 1838).

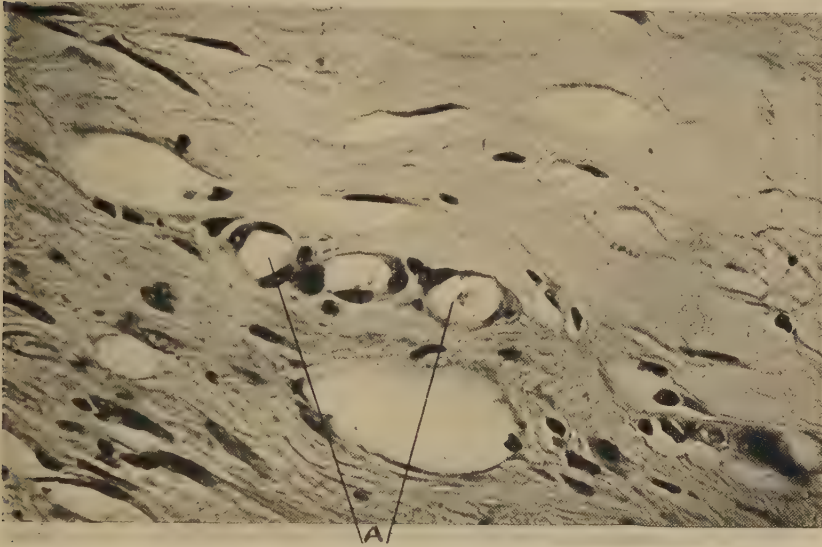


Fig. 5 Photomicrograph, mag. 600. Section of cornea, at pupil. The connective tissue cells (syncytium) surround particles of paraffin. No. 1838. See A.

Microscopic examination: Serial sections through the cornea over the pupil show spaces in the tissue, elongated, oval or round. All these spaces have a distinct outline. They correspond to the situation of the needle-like masses of paraffin in the cornea. Either cells or syncytial masses form definite walls to these spaces (fig. 5). Though the connective tissue cells of the cornea are parallel to its surface, all the cells and syncytial masses bounding the paraffin, conform to the shape of the foreign bodies. In certain of the syncytial masses the nuclei are so numerous and so disposed as to form a structure often noted as a giant cell, of the type known as 'foreign body giant cell.'

This experiment demonstrates that cells become flattened, form syncytium, and adapt themselves to solid foreign bodies; also that giant cells form in tissue purely fibroblastic. Even

though blood vessels appeared in the cornea on the margin of the sclero-corneal junction, microscopically but few capillaries were found in the sections. The left hand opening A, in fig. 5, is undoubtedly a flat synectium around a paraffin mass.

In addition to the experiments in which solid foreign bodies were used, fluids were also tried. The mucous secretion from the gall bladder, free from bile, seemed fitted for such an experiment. The following experiment, No. 4, was tried, therefore, in order to observe the cells that would line a canal, carrying a bland fluid, constructed through the mesodermal tissues of the abdominal wall.

Experiment No. 4. Foreign body fluid. Duration 28 days. Mucous fistula. Dog. No. 162. Operation December 23, 1912. After the cystic duct had been obliterated between two ligatures, a fistula was established from the gall bladder into and through the abdominal wall to a point in the animal's left flank. This biliary fistula was produced by suturing a rubber tube, $\frac{3}{16}$ inch in diameter, into the gall bladder and permitting it to discharge at the skin opening. At the end of five days the tube was removed. There was a copious discharge of sticky mucous which at times was clear, at other times cloudy.

January 20, 1913, second operation. Two inches of tract were excised (Surg. Path. No. 2289) and fixed in Mann's fluid. Celloidin sections were cut across the tract.

Microscopic examination showed that there was a semi-circular opening in the tissue. The stroma around the canal was exceedingly cellular and contained many small blood vessels. In this stroma, polymorphonuclear leucocytes were numerous, and were grouped about the fistulous tract. Within the tract there were also aggregations of leucocytes. The walls of the fistula were distinct and sharply outlined. The cells of what was certainly the walls of the tract in the living animal were large, oval or elongated, and flattened, with large nuclei.

These cells were so arranged that the long axis was parallel to the wall of the tract. The connective tissue cells beneath the surface were more nearly round and were arranged without reference to the wall of the fistula. The surface was almost exclusively cellular, though at isolated points the wall consisted of a 'ground' or intercellular substance. In places, as shown in figures 6 and 7, the lining was a single layer of cells with elongated nuclei. The tissues of the fistular wall was here actually synectial in character since no intercellular margins were made out. Fifty serial sections, eight micra in thickness, demonstrated that closely disposed cells lined a larger part of the canal. At points the lining wall was either debris and leucocytes or coagulated exudate, so massed that the actual structure of the wall could not be made out.



Fig. 6 Photomicrograph, mag. 150. Experiment 28 days. Cross section of walls of mucous fistula showing surface cells. Specimen from subcutaneous tissue of dog. No. 2289. See A. A.

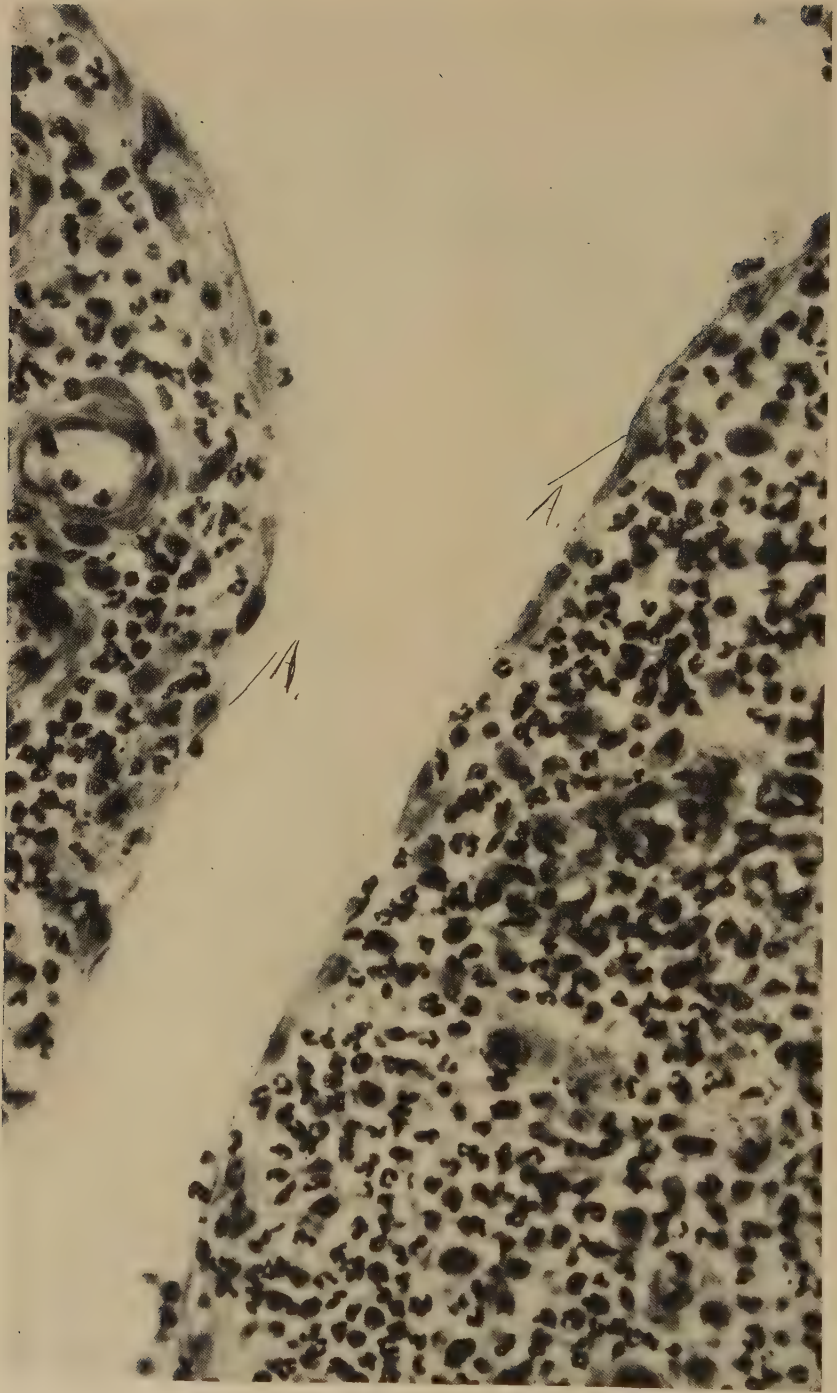


Fig. 7 Mag. 600. Experiment 28 days. Cross section of walls of mucous fistula showing surface cells. Specimen from subcutaneous tissue of dog. No. 2289. See A. A.

In order to obtain a cavity containing serum and not fibrin, 'dead spaces' were studied that frequently obtain where there has been slight inequality in the coaptation of the tissues when operative wounds are closed. Blood clots generally obliterate the greater part of such wound clefts, but the subsequent contraction of the clot and the consequent accumulation of exudate fluid often gives rise to these serum filled spaces. The following operative human case, and experiment No. 4 on a dog, demonstrate such conditions in the tissues, and show the type of lining cells of such cavities.

Case 1. Fluid in contact with tissue seven days. 'Dead space,' Incised wound of thigh. Human adult. Operation by Dr. J. A. Blake, November 21, 1906. Exploratory incision through soft tissues of thigh to the periosteum of the femur. No clinical signs of inflammation developed. Amputation at the hip November 28, 1906, for subperiosteal sarcoma. Specimen (Surg. Path. No. 233) fixed in formalin. Cross section of skin and subcutaneous tissues with exploratory wound revealed a fluid filled space, superficial to deep fascia, about 0.3 cm. in diameter. This space was the result of infolding or puckering of the walls of the wound at the time of the hurried closure. It contained fluid exudate with but little fibrin formation.

Microscopic examination: The wall of the space at points is made up of fibrin and masses of leucocytes. Elsewhere the tissue is thrown into hillocks. The majority of the structural cells are elongated fibroblasts which in numerous places parallel the walls of the dead space. Many of the cells on the surface are elongated fibroblasts, so disposed as to make a definite cellular wall or lining of the space. The stroma beneath the surface consists of large, round, connective tissue cells, with relatively large nuclei at right angles to the plane of the free surface of the space. The actual surface is a layer of syncytium, apparently fibroblastic in character. The nuclei are elongated, stain deeply, are close together in this surface structure, and show no signs of mitotic division. The general appearance of the lining cells is similar to that of the lining cells described in No. 1932 and in No. 2144, shown in figures 8 and 9.

Experiment No. 5. Foreign body: fluid. Duration 7 days. Dog. No. 33. Operation laparotomy, October 2, 1912. Experimental intestinal resection. Uneventful recovery. Autopsy October 9, several hours after death. Gross examination. The abdominal wound was not infected and the healing was by primary union. A section (Surg. Path. No. 2144) across the site of the operative wound demonstrated the irregular but well defined zone leading from the skin through the abdominal wall to the peritoneum, the tract of the healing wound. In the tissues just superficial to the deep fascia there was a cavity or

'dead space' measuring 0.8 by 0.5 cm. and extending along tract of wound. This cavity contained fluid. Specimens were cut from the wall. Paraffin embedding. Microscopic examination. Observation with low power revealed a moderate number of coarse anastomosing strands of fibrin that but partially filled the cavity. Where the fibrin chanced to be attached to the wall of the dead space, the strands extended into the tissues between the cells; as in all healing wounds containing a blood clot. The fibroblasts were directed into the cavity wherever this fibrin served as a support. Where the wall was free from fibrin, sections demonstrated that topographically the surface presented oval or rounded elevations. High power observations of cross sections of the actual surface showed that the cells were large



Fig. 8.

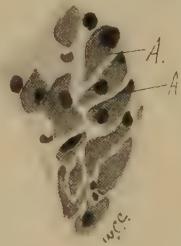


Fig. 9.

Figs. 8 and 9 Duration of experiment 7 days.

Fig. 8 Cross section of wall of 'dead space' showing at A A a complete surface layer of cells.

Fig. 9 Tangential section showing same surface cells, proving that the surface cells are closely apposed and are flattened. See A A.

and elongated, with relatively large, lightly staining nuclei. These cells are tangentially disposed on the free surface of the cavity, and therefore did not reach or point outwards, as did the surface cells at the point where fibrin strands were attached (fig. 8). Sections were cut tangential to the free surface through the wall of the cavity, where it was free from fibrin, to show the surface cells in an opposite plane to that of a cross section. The cells were found closely arranged and varied in shape from oval, elongated, to round; the nuclei were large, elongated and faintly staining, similar to the nuclei of the deeper fibroblasts. Thus it was positively shown that the surface cells were flattened and so disposed as to make a definite cellular covering to the wall (fig. 9). On comparison of a cross section of free surface cells with a tangential section, the cells are seen to be narrower in the plane

parallel to the free surface than in the plane at right angles to the free surface.

The two following experiments are with living tissue grown in the 'observation incubator.' Here it was possible to know absolutely from what tissue the cells were derived that finally enclosed a drop of fluid. In the first experiment, the photograph is of the stained tissue, but in the last experiment the tissue is photographed while alive and unstained.

Experiment No. 6. Cultivation of embryonal tissue in vitro. Drs. McWhorter and Whipple. Tissue from chick of 72 hours' incubation. Specimen (Surg. Path. No. 2374), a small fragment of ectoderm and mesoderm was planted in coagulated plasma. Both the epithelium and the connective tissue proliferated, forming the type of structure shown in figures 10 and 11. As the connective tissue grew and advanced, it was observed encircling a vacuole in the plasma. The serum formed this vacuole as the fibrin around contracted. Since there was no fibrin within the vacuole, the proliferating connective tissue cells met with no support, and reacted to the vacuole as does tissue elsewhere in contact with a non-irritating solid or a fluid foreign body. This specimen was stained en masse and photographed.

Experiment No. 7. Cultivation of tumor tissue in vitro. Drs. McWhorter and Whipple. A small fragment of tissue (Surg. Path. No. 1942) from a sarcoma of a St. Bernard dog was planted in coagulated dog plasma. The connective tissue stroma of the tumor proliferated radially. At one point a vacuole in the mass of coagulated plasma was encountered. This vacuole apparently contained serum which had exuded from the coagulum. Figure 11 of the unstained living tissues demonstrates that the cell structure surrounds the vacuole as a syncytium. The large cell in the centre of the vacuole was moving on the surface of the cover slip, and not on the same plane as the wall of the vacuole. The focal distance of the lens at the magnification of 150 times makes it possible to show both the wall of syncytium enclosing the vacuole and the superimposed cell.

In this change in form of the cells that occurs in the tissues when in contact with a 'foreign body' it is probable that the cells are responding to a physical or chemical reaction in progress in the tissues. In substantiation of this belief the statement of Drew¹ may be quoted, who working with pecten maximus experimentally implanted ovary in muscle tissue. He says

¹ Drew, G. Harold. Experimental Metaplasia. Jour. of Exp. Zool. No. 10, 1911, p. 349.

Briefly summarised my results show that after implantation of a portion of the ripe ovary into the adductor muscle a layer of fibroblasts is formed around it and coincidently the ovarian tissue is invaded by phagocytes and degenerates. After the lapse of about six days no trace or organized ovarian tissue remains, but there is left a cyst surrounded by fibroblasts, and containing blood corpuscles and a

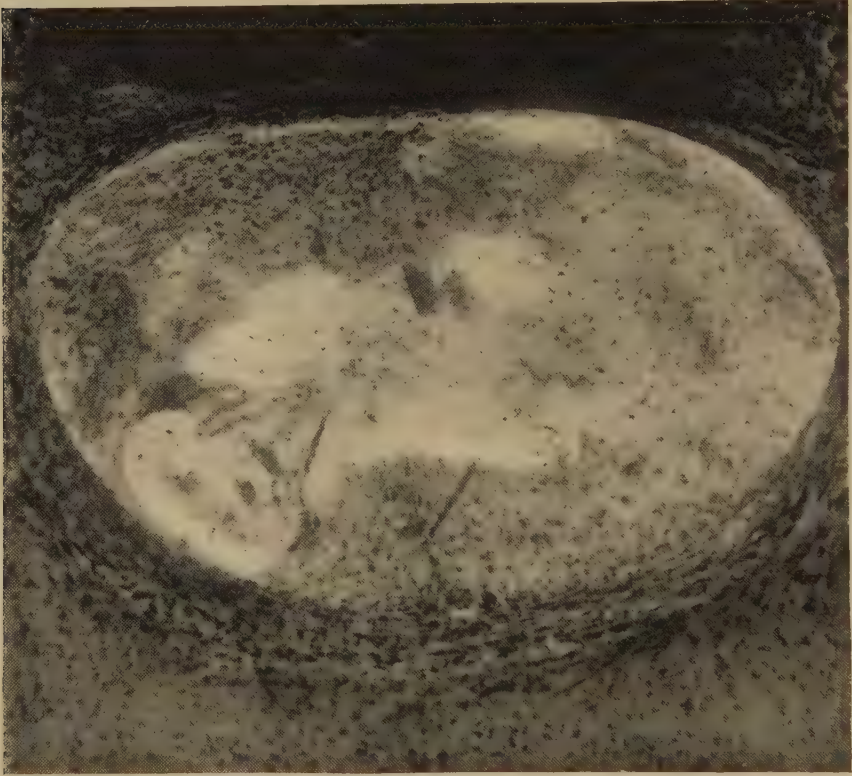


Fig. 10 Photomicrograph, mag. 150. Plant from chick embryo, in vitro culture. Stained mesenchymal cells surround a vacuole in the coagulated plasma. No. 2374.

quantity of small granules having the orange color of the yolk substance. After the lapse of about 20 days more, the innermost fibroblasts gradually change their shape, and form a layer resembling columnar epithelial cells which later become ciliated. Eventually the whole cyst becomes lined with well defined ciliated epithelium, which persists at least for 120 days, which is the longest period I have yet succeeded in keeping the animals alive in the experimental tanks. I

consider that there is some evidence to show that this change of the fibroblasts into ciliated epithelium is a reaction to the presence of some definite chemical substance within the cyst.

He further believes the fibroblasts of the implanted mass die and that the cyst lining is formed from the fibroblasts of the site of injection. He undertakes experiments to prove that the lining



Fig. 11 Photomicrograph, mag. 150. In vitro culture. Unstained growing tissue. The connective tissue cells surround a vacuole that occurred in the culture media. (Coagulated plasma) No. 1942.

cells of the cyst have not originated from the ciliated cells of the transplanted oviduct, but from the cells of the host, at the site of injection.

Drew and DeMorgan² performed some experiments in *Pecten maximus* to determine the origin and method of formation of the

² Drew, G. Harold and De Morgan, W. The origin and formation of fibrous tissue produced as a reaction to injury in *Pecten Maximus*. *Quart. Jour. Micro. Sci.*, vol. 55, part 3.

fibrous tissue formed as a capsule or wall about foreign bodies. They believe these structures arise from the fibroblasts in the walls of blood spaces and in the intermuscular connective tissue. The cells divide, migrate and arrange themselves in a concentric manner about the foreign body. These fibroblasts thicken and later present a somewhat stratified appearance. These observers believe the reaction to injury in the animal forms used is exactly similar to that which occurs in vertebrates.

In conclusion it may be stated the subcutaneous connective tissues react to the presence of a smooth surfaced, non-irritating foreign body, i.e., celloidine, in such a manner that there results a distinct pavement layer of flattened cells, the outlines of which are demonstrable by the impregnation of their intercellular substance with a silver salt. The tissues of the cornea, free from blood vessels, in contact with paraffin form similar flattened cells. Cells that are flattened and arranged to form a definite wall, lined the mucous fistula and the 'dead spaces' containing serum. It should be observed that all these results occurred in the connective tissue stroma of the structures injured by the experiment. Drew and DeMorgan obtained a reaction in the connective tissue stroma when foreign bodies had been introduced. This encapsulation of foreign bodies by connective tissue is a well known reaction of the tissues. The experiments with living tissues in the 'observation incubator' showed a similar cellular lining in the vacuoles in the media of coagulated plasma.

The above experiments show after repair is complete that the free surface cells of accidental spaces in the tissues are flattened and form a pavement. In other words after an injury, the connective tissue cells that are exposed become changed in the above manner. Therefore the second hypothesis premised in this article is tenable, namely, that the flat cells of serous surfaces and those lining blood vessels may regenerate from deep connective tissue cells and do not necessarily arise from adjacent intact mesothelial or endothelial cells.

A STUDY OF THE MORPHOLOGY OF THE INFERIOR OLIVE

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SIX FIGURES

At the suggestion of Dr. Mall I am making a preliminary report on the morphology of the inferior olivary nucleus and its accessory nuclei; the portion of the work embodied in this report was completed in the laboratory of Anatomy at Johns Hopkins University in 1912 and 1913, the subjects used for this study were selected from Dr. Mall's collection of human embryos.

This work was undertaken in the attempt to establish as definitely as possible, the external form and configuration of these nuclear masses and their relations to each other, as a preliminary to a further study of the human brain-stem.

A study of these masses, as found in the new-born, has already been included by Miss Sabin, in her comprehensive work on the Medulla and Midbrain¹ and a careful presentation of the adult type by Weed,² both workers having conducted their researches in this laboratory. So it was determined to use, in this instance, an earlier stage of development as a comparison to ascertain, what, if any differences, would be manifested in the various ages. Acting upon the advice of Dr. Essick, who was familiar with the Mall collection, Embryo No. 491 was chosen for this study, this specimen was a transsection of the brain-stem of a 280 mm. (CR) fetus, that had been cut, serially 40 micra thick and stained with haematoxylin, and which offered a clear cellular

¹ An atlas of the medulla and midbrain, by Florence R. Sabin, M.D., published by the Friedenwald Co., Baltimore, Md.

² A reconstruction of the nuclear masses in the lower portion of the human brain-stem, by Lewis H. Weed, M.D. Publication No. 191 of the Carnegie Institution of Washington.

differentiation, and such a stage of development as not to differ too widely from the subjects used by the other workers mentioned. This specimen was studied and modelled as described below; but since it only presented the results as seen upon cross-section, as did the work of both investigators mentioned, I planned to make a series of reconstructions, one in each plane, with the double purpose of gaining a more comprehensive knowledge of these nuclei, and at the same time checking up the accuracy of my work and the more certainly approximating the true form and appearance of the nuclei. Accordingly two other feti were selected, viz., No. 619, which was 200 mm. crown rump length; and No. 625 which was 220 mm. crown rump length. Both were chosen because they presented the nearest measurements to the original selection obtainable, the brain stem was removed, hardened, embedded in paraffin and sectioned serially 40 micra thick, in each instance. No. 619 was cut in the sagittal plane, No. 625 in the coronal plane, and both were stained with haematoxylin and counterstained with erythrosin. These were then carried through the same steps as was No. 491, every other section was selected and marked, projected, traced, cut and piled in the same way as the original model, thus reconstructions of the nuclei were secured representing all three planes and a sufficient similarity in results was obtained to warrant the assumption that the original model was an accurate representation of the nuclei.

In the study and reconstruction of Embryo No. 491 every other section was drawn at a magnification of twenty-five diameters throughout the limits of the nuclei. In making each drawing the left half of each section was traced, extending the peripheral lines well beyond the median raphe the median line was carefully marked; a piece of stiff cardboard was cut 2 cm. in width, with parallel straight edges, one edge was fitted along the median line, as marked on the tracing, along the other edge a line was drawn, thus making a straight-edge to pile by, each drawing was controlled by a comparative study with a higher magnification. Following this the drawings were traced upon wax plates, 2 mm. in thickness. Every fifth tracing was made upon

a plate in which lamp-black had been incorporated with the wax mixture, each plate was then carefully cut around the periphery and numbered in serial order; (this method of reconstruction was first recommended by Born, and has been described by Bardeen³ and others as followed in this laboratory.

Having completed the tracings, a wooden form was built with one perpendicular, flat surface accurately squared with its base; upon this upright surface two parallel, perpendicular lines were drawn 10 cm. apart.

After all the plates had been cut out, they were piled in numerical order, orienting by the external form and by squaring the straight edges, which in the completed pile formed a flat surface. This side was then squared with the corresponding surface of the wooden upright and marked with two parallel cuts to correspond to the lines which had been drawn upon the wooden form. Following this, the nuclei were cut out in the first plate, leaving 'bridges' connecting the masses to the surrounding wax, and a plate fixed upon a glass slab. The wooden form was then placed against the straight edge so that the lines upon the box fitted against the cuts upon the wax plate, the base of the box indelibly outlined upon the glass slab and the box removed. After each succeeding plate had been cut and piled, orienting by the outline configuration, the form was placed in its marked position to complete the orientation, thus overcoming any tendency to 'pull' the model one way or another. The form was then removed and the plate fixed to the pile with a hot knife, pins being used after a sufficient number of plates had been piled to afford the necessary bulk; as the plates were piled cuts were made, front and back, so that a portion of the shell could be removed and the olive modelled to better advantage. This procedure was controlled by drawing a line across each side where the cut was to be made then in replacing the piece it could be fitted accurately by reCompleting the broken lines. These methods were followed carefully throughout the

³ Born's method of reconstruction by means of wax plates as used in the Anatomical Laboratory of the Johns Hopkins University, by Charles Russell Bardeen. Johns Hopkins Hospital Bulletin, vol. 12, 1901.

reconstruction. Frequent comparisons were also made, both with the tracings and with the section from which they were made. After completing the reconstruction, the model of the main nucleus was removed and studied in toto, the accessory nuclei had been anchored to the shell in order to separate them from the main nucleus when it was removed. It was found that the cephalic extremity of the ventromesial plate of the accessory nucleus was continuous with the upper part of the ventral lip of the main nucleus. This connection had to be cut through before the olive could be removed from the shell. This same point of fusion was noted in all three reconstructions.

The completed model of the main nucleus (figs. 1, 2 and 3) is a flattened oblong mass, presenting ventromesial and dorso-lateral surfaces, dorsomesial and ventrolateral borders, and upper or cerebral, and lower or spinal extremities; its greatest vertical measurement is 210 mm., its greatest width is 204 mm., and its greatest thickness 64 mm.

The ventromesial surface is convex in outline, both vertically and transversely, and narrows considerably in its lower three-fourths, due to the encroachment of the hilus upon the ventral lip of the dorsomesial border. In the upper portion the two lips of the hilus are parallel for approximately a fourth of the way down, below this point the ventral lip recedes gradually, thus narrowing this surface below.

The dorsolateral surface is flattened in outline, though very irregular, as the convolutions and sulci are more marked upon this surface than elsewhere. This surface is more extensive than the ventromesial, and is quadrilateral in shape.

The ventrolateral border is broad, rounded and marked by the alternate ridges and depressions of the obliquely directed convolutions and sulci; this border, extending lateral to the pyramid forms the prominence on the ventrolateral aspect of the medulla, the olivary eminence. The rootlets of the hypoglossal nerve, pass first, between the hilus of the main nucleus and the accessory bodies, then curve lateralward passing between the pyramid and the ventromesial surface of the olive to appear superficially along the groove that separates the olivary eminence

from the pyramid, marking the position of the ventral margin of the ventrolateral border. This border is continuous with the surfaces along its rounded margins.



Fig. 1 Shows the ventromesial surface. Note the narrowing due to the deficient ventral lip of the hilus. *H*, marks the superior and *T*, the inferior extremities. *M*, the dorsomesial and *L*, the ventrolateral borders. All the figures are from the olive of specimen No. 491 and they are enlarged $12\frac{1}{2}$ diam.

The dorsomesial border, thinner than the ventrolateral, is the site of the hilus, which is a long, narrow, slit like opening between the leaflets, occupying almost the entire extent of this border. The hilus looks dorsomedialward and is embraced by the curv-

ing mass of the accessory nuclei. It is bounded by a prominent, rounded dorsal lip, which is practically a straight-edge, and is marked by numerous transverse elevations and depressions. The depressions are continuous with the tortuous sulci



Fig. 2 Shows the ventrolateral border, *H*, marks the superior and *T*, the inferior extremities; *V*, the ventromesial and *D*, the dorsolateral surfaces.

of the interior, and the elevations correspond more closely with the convolutions of the exterior, than with those upon the interior. This arrangement leaves a continuous, fairly smooth margin along the more dorsal portion of this lip. The ventral

lip is prominent and parallel to the dorsal lip for some 50 mm. below the cephalic extremity, from this point the lip becomes thin and gradually recedes until it reaches a plane 42 mm. lateral to the plane of the dorsal lip. This widest measurement is found



Fig. 3. Shows the dorsolateral surface. The lettering is the same as figure 1.

40 mm. above the lower extremity of the olive. From this point to the lower end of the hilus, the lip curves slightly medialward. The ventral lip, in its upper, prominent portion is similar in appearance to the dorsal lip, from this point downwards

it is thin and fairly smooth upon both its internal and external surfaces along a narrow rim, exhibiting but little tendency to conform to the wrinkling so noticeable in the rest of the surface. At the point named on this border, 50 mm. below its cephalic extremity, it was continuous with the upper end of the ventromesial plate of the accessory bodies. Between the olive and the ventromesial plate, the intermediate accessory plate is interposed, this plate is narrow above at the point of fusion and is separated from the ventral lip of the main nucleus by a narrow interval. The width of this interval remains comparatively unchanged throughout so that the receding ventral lip is compensated for by the widening intermediate accessory plate. A study of a younger specimen, modelled at a later date than the subject of this report, shows the ventral lip of the hilus to be drawn medialward and united with the remainder of the ventromesial leaflet along a much thinned line, this line of tenuity corresponding to the future interval between the receding ventral lip and the lateral edge of the intermediate accessory mass. The fibre bundles of the hypoglossal nerve, which are increasingly prominent along the level of the lower three-fourths of the olive, pass through this interval in their lateralward curve and probably cut away this accessory plate from the ventral lip of the hilus. The hilus extends from a point 24 mm. below the highest part of the upper extremity, to a point 22 mm. above the lowest part of the lower extremity of the main nucleus.

Both extremities are blunt and rounded, the inferior being less extensive than the superior, owing to the deficiency of the ventral lip below, where so much of the main nucleus has been sacrificed to form the accessory bodies. Both extremities present deep clefts and prominent elevations which are continuous with the gyri and sulci upon the surfaces, those of the lower extremity being more pronounced though less numerous than those of the upper.

The greater portion of the caudal extremity of the main nucleus is covered by the accessory bodies, which extend considerably lower in the brain-stem than is the case with the main nucleus.

The general form of the olive, is, as Miss Sabin has so aptly described it, "that of a hollow shell with a wrinkled wall;" the thinness of the shell being such that each external depression causes a corresponding elevation upon the interior, where the intervening depressions produce in turn the external convolutions; the tortuous outline on cross section is very marked as shown in figure 4 (which is from 491-16-3-1).

In describing the outline configuration of this nucleus, whilst there is a noticeable general similarity, certain features which are fairly constant, there is no hard and fast rule which will apply in every instance, since each nucleus presents certain modifi-

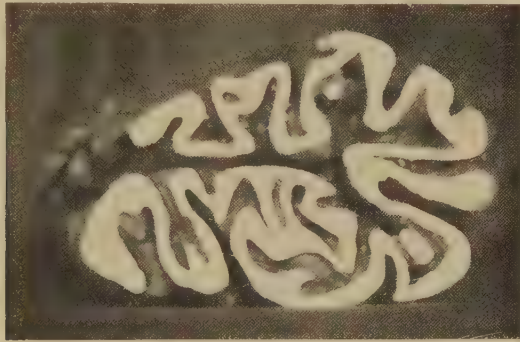


Fig. 4 Emb. 491, slide 16, row 3, section No. 1 and shows the complicated pattern on cross-section.

cations of the general plan which are peculiar to itself. All of the inferior olivary nuclei, after an advanced stage of development is reached, present a complex pattern, due to the tortuous windings of its elevations and depressions, which have been more or less appropriately compared to those of the cerebral cortex, and like the cerebral pattern, it differs not only in different subjects, but also upon the two sides of the same subject, consequently the minute and detailed descriptions based upon the study of reconstructions are only to be considered as applying with any considerable degree of accuracy to the one model which has been the subject of the study. The following description is that of the model of Embryo No. 491, which was approximately

an eight months fetus, and consequently, reasonably close, as to size and degree of development, to that of the new-born, and possibly not differing very widely from the adult type.

One marked difference between the cerebral pattern and that of the olive is, that due to the thinness of the wall of the nucleus, every marking upon the exterior has its complement upon the interior. The reverse being equally true, this fact presents an additional difficulty in the attempt to subdivide the surface of the olive into definitely outlined areas.

The markings upon the ventromesial surface and the ventrolateral border, are, for the most part, arranged as folds with intervening sulci, both of which are directed obliquely upward and lateralward from the ventral lip of the hilus. Upon reaching the dorsal margin of the ventrolateral border, they take a general transverse course across the dorsolateral surface toward the dorsal lip of the hilus, upon this surface though the sulci are deeper, more irregular and present numerous branches, and the folds or convolutions are more tortuous, being much broken by sulci, both by the main ones and their branches and by smaller unconnected ones, thus rendering the pattern more complex and more difficult to follow. In order to trace the pattern to better advantage, I had the entire exterior of the olive drawn as a continuous flat surface, just as one would open a book and lay it face down in order to gain a complete view of its backs (fig. 5). Reading from left to right with the caudal extremity of the nucleus toward you, there will be seen to be eight fairly continuous convolutions, separated by seven main sulci. The first sulcus is limited to the dorsolateral surface, beginning well forward upon the lower extremity. At the lateral margin of the surface it passes obliquely upward and medialward to the middle of this surface, then turns directly upward to end beneath an annectent gyrus which connects the first and second convolutions at a point a little above the junction of the middle and lower thirds of the olive. A small branch is given off from the medial side of this sulcus which curves upward in the first convolution. The first sulcus separates the first convolution from the second, save at the point mentioned. The second sulcus begins on the ven-

tromesial surface, well back from the hilus, and passes obliquely upward and lateralward, then curving around the ventrolateral border passes upward and medialward, to end in a transverse fissure on the dorsolateral surface. This latter fissure limits superiorly, the annectent gyrus which connects the first and second convolutions, the second sulcus separates the second con-

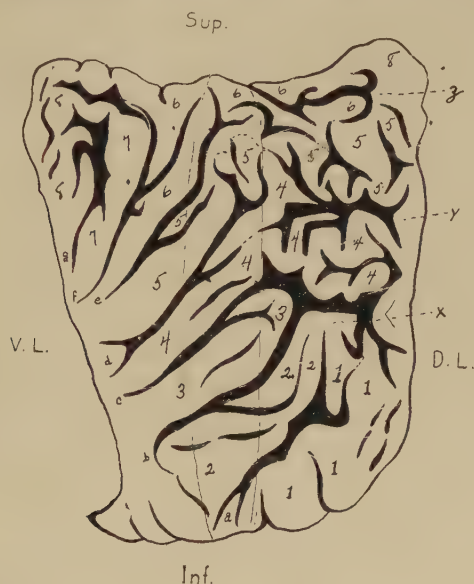


Fig. 5 The plane drawing of the entire exterior of the olive, the numerals 1 to 8 inclusive mark the convolutions; the letters *a* to *g* inclusive mark the sulci, *x*, *y* and *z* mark the first, second and third transverse fissures upon the dorsolateral surface. *Sup.* marks the upper and *Inf.* the lower extremities; *V.L.* the ventral and *D.L.* the dorsal lips of the hilus. The continuous vertical lines indicate the margins of the ventrolateral border.

volution from the third. The third sulcus begins upon the ventromesial surface just short of the ventral lip passing obliquely upward and lateralward curves around the ventrolateral border with a marked upward trend, to end in the transverse fissure on the dorsolateral surface. A collateral is given off from the third sulcus which ascends and is lost in the third convolution. The third sulcus separates the third convolution

from the fourth. The transverse fissure on the dorsolateral surface is very deep and extends well across this surface a little below its middle. It receives the second and third sulci and separates the third, and the connected first and second convolutions from the fourth. The fourth sulcus has the same course as the third until it reaches the middle of the ventrolateral border, where it divides into an upper and a lower limb, the upper limb is better defined and has a more marked upward trend. At the dorsal margin of this border it turns sharply downward and medialward to join the distal extremity of the lower limb in a second transverse fissure; the lower limb of the fourth sulcus, less distinct than the upper, pursues the same general course, but is almost effaced where it turns over the dorsal margin of the ventrolateral border, becoming more distinct on the dorsolateral surface, where it joins the upper limb in the second transverse fissure. The fourth sulcus and its transverse portion, separate the fourth convolution from the fifth. The second transverse fissure, really only the continuation of the united distal extremities of the two limbs of the fourth sulcus, is more broken than the first transverse fissure, and extends entirely across the dorsolateral surface at the junction of its upper and middle thirds. The fifth sulcus begins like the preceding one and presents a more marked upward trend. At the ventral margin of the ventrolateral border it gives off a lower limb which ascends and is lost in the fifth convolution, the main sulcus continuing obliquely upward and dorsalward to near the upper extremity of the olive, where it divides into a 'U' shaped sulcus, the short limbs of which embrace the curve of an annectent gyrus which connects portions of the fifth and sixth convolutions, the main portion of the sulcus separating these two convolutions. Upon the dorsolateral surface there is a secondary sulcus which has a downward and medialward course from the annectent gyrus mentioned, across the center of which a faint line apparently connects this sulcus with the fifth, this accessory sulcus completes the separation of the fifth and sixth convolutions. The sixth sulcus begins almost at the margin of the ventral lip and is confined to the ventromesial surface. It ascends with only a slight

lateralward inclination. At the junction of the upper and middle thirds of the olive this sulcus gives off a branch which ascends obliquely in the sixth convolution, curving on to the ventrolateral border to end near the upper extremity of the olive in a faint connection with the ventral limb of the 'U' of the fifth sulcus. The main limb of the sixth sulcus ascends to near the upper portion of the ventromesial surface, where it unites with a transverse fissure; the main limb of the sixth sulcus and the transverse fissure separate the sixth convolution from the seventh. The transverse fissure marks the margin delimiting the upper extremity from the ventromesial surface, it is deep and irregular and continuous laterally with the main limb of the sixth sulcus. The seventh sulcus is deep and irregular, and pursues an almost vertical course from near the middle point of the ventral lip, to an annectent gyrus which connects the seventh and eighth convolutions. This gyrus separates the sulcus from the transverse fissure, and gives off from its medial side, a lower deep, and an upper shallow collateral sulcus which mark the eighth convolution. The seventh sulcus partially separates the seventh convolution from the eighth. Beginning well forward on the upper portion of the ventrolateral border is a deep cut which crosses the upper extremity and runs downward and medialward upon the dorsolateral surface, becoming irregular, and sending off numerous side branches. This sulcus at its beginning, splits the upper end of the sixth convolution, and ends beneath an overhanging prominence on the medial portion of the dorsomesial border. This prominence is continuous, over the upper end of the hilus, with the eighth convolution, and has been considered as a portion of that fold; the sulcus thus subdivides the sixth convolution and separates a portion of that convolution from the eighth.

The first convolution is irregular, marked by numerous short, deep depressions, rather semilunar in shape and includes almost the whole of the lower extremity and approximately the lower third of the dorsal lip of the hilus, and is separated from the second convolution by the first sulcus save at its two extremities, where it is connected by annectent gyri with the second convolu-

tion. The second convolution begins upon the lower extremity and ventrolateral border, where it is connected to the first convolution, it then turns upward upon the dorsolateral surface to end below the first transverse fissure by connecting with the superior extremity of the first convolution. This second fold is less irregular than the first, and is subdivided on the dorsolateral surface by a collateral of the second sulcus, and is bounded by the first and the main limb of the second sulcus upon its two sides, and by the first transverse fissure above. The third convolution is smaller than its predecessors. It is but faintly represented upon the ventromesial surface prominent on the ventrolateral border, and ends in a blunt, rounded extremity upon the dorsolateral surface. It is subdivided into three rolls by a fairly well-defined secondary sulcus below, and by the lower limb of the third sulcus above; separated from the second convolution by the deep second sulcus—which ends in the transverse fissure above—and from the fourth convolution by the main limb of the third sulcus. This limb turns around the upper extremity of the convolution to end in the transverse fissure thus sharply outlining this fold. The fourth convolution begins well forward upon the ventromesial surface, ascends obliquely, curving over the ventrolateral border to extend at first slightly downward, then transversely upon the dorsolateral surface. It is bounded by the third and fourth sulci and the first and second transverse fissures. At its beginning it is fairly smooth, being partially subdivided above by the lower limb of the fourth sulcus, but upon the dorsolateral surface it is broken and subdivided by secondary depressions into four irregular, connected folds. The fifth convolution presents a marked similarity to the preceding one, beginning as a fairly smooth prominence upon the ventromesial surface, it has a more marked upward trend than the fourth, and is broken above by a collateral from the fifth sulcus. It then becomes irregular in outline and curving over the dorsal margin of the ventrolateral border turns sharply downward, then transversely across the dorsolateral surface, where it becomes still more irregular, being broken into smaller folds by the offshoots from the second transverse fissure below, and from a secondary

fissure above, this convolution is connected to the sixth near the upper extremity of the ventrolateral border by an annectent gyrus. The fifth convolution is bounded below by the fourth sulcus and the second transverse fissure, above, by the fifth sulcus and the secondary sulcus. The sixth convolution beginning upon the ventromesial surface passes almost directly upward to the upper extremity of the olive where it is split into two portions, the more ventral of which extends medially along the upper extremity to join the eighth convolution. The more dorsal fold turns sharply downward upon the dorsolateral surface, at the middle of which it turns upon itself to end in a rounded extremity beneath the overhanging eighth convolution. The sixth convolution is bounded by the fifth sulcus and the secondary sulcus previously described, above, by the sixth sulcus and the transverse fissure upon the upper extremity of the ventromesial surface. The seventh convolution is a narrow fold, limited entirely to the ventromesial surface. Beginning at the middle of the olive well forward upon the ventral lip, it ascends with a medial curve to the upper extremity of the surface where it is joined to the eighth convolution by an annectent gyrus just below the transverse fissure. This convolution is comparatively smooth, and is bounded laterally by the sixth and mesially by the seventh sulci and is limited above by the transverse fissure and the sixth sulcus at their junction, where it is connected to the eighth fold. The eighth convolution is of triangular shape and forms the mesial half of the ventromesial surface of the olive above. It is bounded laterally by the seventh sulcus, medially by the ventral lip of the hilus. At the upper extremity of the olive, of which it forms the highest part, it curves over on to the dorsolateral surface above, forming slightly more than the upper fifth of the dorsal lip of the hilus. The main mass of the convolution is very irregular where the tortuous collaterals of the seventh sulcus mark its surface. It is connected to both the sixth and seventh convolution by annectent gyri, the former appearing upon the upper extremity, the latter upon the ventromesial surface.

To gain an adequate conception of the wrinkling of the surface of the olive, it is necessary to study the individual sections

themselves (fig. 4). These differ in complexity not only with the varying changes in the nuclear outline, but with the degree of shortening of the ventral lip. This shortening, most apparent in the lower levels, occurs upon the ventromesial surface, which is



Fig. 6 Drawing of the accessory nuclei, *I* is the inferior and *S* the superior extremities. *V.P.* the ventromesial plate, *I.P.*, the intermediate plate, and *D.P.* the dorsal plate, *X* marks the position of the lower extremity of the olive, *P.F.* the point of fusion of the ventromesial plate with the ventral lip of the hilus of the main nucleus.

applied against the dorsum of the pyramid, being separated from that tract by the accessory nuclei.

The accessory bodies (fig. 6) are irregular, flattened plates, and are not wrinkled as is the case with the surface of the main nucleus. Collectively they consist of three plates, more or less

fused in such manner as to embrace and form an incomplete covering for the hilus of the main nucleus. The combined mass of the accessory nuclei has the same vertical measurement as the main nucleus, viz., 210 mm. long, beginning above 34 mm. below the upper extremity of the olive and extending 34 mm. below the lower extremity, this lower extension of the accessory bodies is an irregular, fused mass presenting two isolated particles. One, the larger, is 41 mm. long, rounded in shape and situated upon the dorsolateral aspect of the olive, in line with and 27 mm. below the narrowed lower extremity of the dorsal plate. The other is a small, isolated nodule placed lateral to the mass. The main portion of the mass below the olive lays to the ventromesial side and is continuous above with both the ventromesial and intermediate plates.

The three plates which make up the mass of the accessory nuclei may be named from their relation to the dorsomesial border of the olive, ventromesial, intermediate and dorsal. The ventromesial plate is a long, narrow mass, flattened from without inward and is separated from the olive by the intermediate plate. The ventromesial plate is continuous below with the bulky lower mass of the intermediate plate passing upward. It crosses mesial to the intermediate plate to become continuous along its dorsal margin with the upper part of the dorsal plate, and on its ventral margin becomes continuous with the ventral lip of the hilus of the main nucleus as mentioned. The intermediate plate, broad and thick, forms the bulky mass which extends below the main nucleus, extending upward ventromesial to the olive. This plate occupies the site of the deficient ventral lip of the hilus, being separated from the main nucleus by the hypoglossal fibres, as is also the ventromesial plate. Extending upward the intermediate plate is again fused on its mesial surface with the ventromesial plate above, and is joined by a narrow bridge, to the mesial margin of the dorsal plate. The intermediate plate forms the greater bulk of the accessory bodies. The dorsal plate begins farther cerebralward than the remainder of the accessory nuclei, and from this point extends downward as a broad, thin lamina

which curves up over the dorsolateral surface of the olive. It is continuous at the upper part of its mesial margin with the ventromesial plate, and opposite the middle of the main olive is connected with the intermediate plate by an oblique bridge. This plate narrows at its lower extremity, the apex being in line with the isolated mass below.

Both the main accessory mass, as described, and the small associated bodies, present the same structural elements as are found making up the main nucleus.

HERNIA OF THE SMALL INTESTINE INTO A PERSISTENT GREAT OMENTAL CAVITY

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In the regular course of dissection in the Anatomical laboratory of the University of Michigan there was noted by the writer a subject in which after opening the abdominal cavity—with parts still in situ—it was observed that the greater part of the small intestine was lodged in a persistent cavity of the great omentum; entrance to which had been gained by an opening in the left half of the transverse mesocolon in the region between the greater curvature of the stomach and the transverse colon. The rarity of this observation induced a search in the literature pertaining to similar conditions and it was found that relatively few cases presenting like relations and position of the intestines have been placed on record. A search of the literature was made difficult by view of the fact that the recorded cases are listed under various titles, such as internal hernias, hernia bursa omentalis, hernia Foramen of Winslow, hernia intermesocolica, Treitz hernia, etc. The author thinks that these cases should be called hernias of the bursa omentalis.

Since all cases of more marked deviations from the normal in the position of the different parts of the alimentary canal and the mesenteries has an interest both from the developmental and from the gross anatomical view points, the latter more particularly with reference to the diagnosis of pathological conditions and to surgery of the intestine, the case under consideration seemed worthy of record. The pertinent literature is given in very brief review and this before the case in question is presented.

By way of a very general summary, it may be stated that in only five of the recorded cases was a persistent cavity of the great

omentum noted; in four others, a partial separation of the adherent layers of the great omentum was mentioned; in twenty-three of the recorded cases, the hernia entered the bursa omentalis by way of the epiploic foramen; in the same number of cases through openings in the transverse mesocolon; in three cases through openings in the walls of the great omentum; and in one case through a hiatus in the gastro-hepatic omentum. The small intestine was the viscus producing the hernia in forty-two cases, portions of the large intestine in three cases, and portions of both large and small intestines in five cases.

The cases recorded in the literature may be grouped into the following subdivisions of Hernia of the bursa omentalis:

a) Herniae entering the bursa omentalis through the foramen epiploic.

b) Herniae entering by way of an opening in the transverse mesocolon.

c) Herniae through openings in the walls of the great omentum.

d) Herniae entering the bursa omentalis by way of an opening in the gastro-hepatic omentum.

The first record of a case in which the hernia entered through the epiploic foramen was made by Blandin ('34). He observed at an autopsy a large segment of the small intestine in an opening in the transverse mesocolon, its point of exit from the bursa omentalis. These loops of intestine had entered the bursa omentalis through the epiploic foramen. Rokitansky ('42) reported having seen a large portion of the small intestine strangulated in the epiploic foramen. Treitz ('57) records a case in which two loops of jejunum were found in the lesser peritoneal sac, which was entered through the epiploic foramen. He stated that he had often seen parts of the large intestine, especially a hepatic flexure or abnormal loop of the transverse colon in this foramen. J. Wilson Moir ('68), as quoted by Cheine, observed most of the small intestine within the lesser sac having passed in through the epiploic foramen. Novello ('81) reported a case where two meters of small intestine had passed through the epiploic foramen into the bursa omentalis. Mojoli ('84) observed a loop of the transverse colon in the epiploic opening. Elliott-Square ('86) found at an operation "eight inches of ileum, two feet from its termination," in the epiploic foramen. This foramen was large enough to admit two fingers. Treves ('88) reported an operation at which he found the caecum, ascending, part of the transverse colon, and between two and three feet of ileum, had passed through the epiploic foramen. He noticed that the caecum was of the undescended type, and had led the hernia into the lesser sac. A part of the hernia reappeared in the greater sac through an opening in the gastro-hepatic omentum. The appendix was found at the lesser

curvature of the stomach, near the oesophagus. Gangolphe ('90) found the greater part of the small intestine within the bursa omentalis which it had reached by way of the epiploic foramen. Neve ('92) observed at an operation that the ascending and part of the transverse colon had passed through a ring situated behind the stomach and to the right of the median line. It was large enough to admit two fingers, but he believed it to be the epiploic foramen. Rehn ('92) reports having seen parts of the small intestine in the epiploic foramen. Picardo ('93) saw a case where part of the small intestine was herniated through the epiploic foramen into the lesser sac. Stecchi ('94) had a case with small intestine coils within the small peritoneal sac which they had reached by way of the epiploic opening. Reynier ('97) writes of a case operated on by Dr. Wall and himself in which they found the greater part of the small intestine within the bursa omentalis. The epiploic foramen was the passage way for the hernia. Mori ('98) observed a case where part of the colon had passed through the epiploic foramen into the lesser sac. Rawitsch-Schtscherbo ('99) found in their case a diverticulum of the small intestine, nine centimeters long, in the foramen of Winslow. Groves and Martin ('00) had a case where parts of both small and large intestine were in a bursa omentalis hernia, entering by way of the foramen of Winslow. Adajaroff ('01) reported a strangulation of part of the small intestine in the epiploic foramen. Jeanbrau and Riche ('02) had a case where the duodeno-jejunal angle was the part in the epiploic foramen. Delkeskamp ('05) reports a case of a woman who after normal labor developed intestinal obstruction. At operation, it was found that all of the large intestine, except the sigmoid colon, and also coils of the small intestine had passed through the epiploic foramen into a "peritoneal sac of the shape of a dilated stomach." He found a common ileo-caecal mesentery and a very long sigmoid mesocolon. Carwardine ('08) writes of a case in which he found two and a half feet of ileum forming a bursa omentalis hernia by passing through the epiploic foramen. Borszéký ('12) reported a case in which he found part of the small intestine passing through the epiploic foramen into the lesser sac and out through a slit in the gastro-hepatic omentum into the greater sac.

Of the recorded cases of hernia of the bursa omentalis in which entrance to the lesser sac was found through an opening in the transverse mesocolon, Loeb ('44) was the first reporter. He observed an opening the size of the fist about the middle of the transverse mesocolon through which the greater part of the small intestine had passed into the "cavity of the great omentum." Diettrich ('47) reported a case in which there was an opening in the transverse mesocolon through which "all of the movable small intestine had passed into the bursa and out again through an opening in the omentum magnus (ligament gastro-colic) so that they hung in front of the transverse colon and free part of the great omentum." Deville ('51) observed a case in which an opening in the transverse mesocolon was found opposite to the body of the fourth lumbar vertebra. Through this had passed

coils of the small intestine into the lesser sac. The transverse colon descended as a sling to the level of the pelvis. Treitz ('57) reported two cases with openings in the transverse mesocolon through which the greater parts of the jejunums had reached the lesser sacs and lay between the layers of the great omenta. Rembold ('65) saw in a case an opening in the transverse mesocolon through which two and a half feet of small intestine had passed into a space "between the liver and an hour-glass stomach" (bursa). Boettcher ('76) in a case found an opening larger than the size of a fist in the transverse mesocolon. It had smooth borders, and was bounded by blood vessels. Most of the small intestine had passed through into the bursa, and lay between the layers of the free part of the great omentum—persistent great omental cavity. Furst ('94) reported a case with a nine centimeter opening in the transverse mesocolon through which one-third of the small intestine had entered the bursa. Hakonson ('94) saw in a case of hour-glass stomach great defects in the transverse mesocolon and gastro-hepatic omentum. Through the first of these openings, part of the colon and coils of the small intestine had entered the lesser peritoneal sac and returned to the greater sac through the second opening to hang down in front of the stomach. Laurer ('94) writes of a case possessing similar openings. The patient had an hour-glass stomach. Part of the small intestine passed through an opening in the transverse mesocolon and out of the bursa by means of the opening in the lesser omentum. Sundberg ('97) reports a case with a defect of the transverse mesocolon measuring five by ten centimeters. Through this the jejunum and most of the ileum entered the omental bursa. Here again the coils reappeared in the greater peritoneal sac through an opening in the lesser omentum. Akerman ('02) saw an opening the size of a fist in the transverse mesocolon of a patient at an operation. The greater part of the ileum, the caecum, and the lower end of the ascending colon had passed through this opening into the omental bursa and then part of the hernia returned to the greater sac by means of the epiploic foramen. Narath ('93) names the opening into the bursa in the case recorded by him the "recessus duodeno-jejunalis." Schumacher suggests designating it "the radix of the mesocolon transversum." Most of the coils of the small intestine passed through this opening into the bursa omentalis, and then reappeared in an opening in the gastro-hepatic omentum, certain of the coils hanging in front of the stomach. Bastianelli ('04) describes a case of retroposition of the colon. The short transverse mesocolon had an opening through which a coil of the small intestine had passed. Schwalbe ('04) found in a subject at autopsy a five centimeter opening in the transverse mesocolon through which most of the small intestine had passed into the omental bursa. Two coils occupied a separation of the layers of the great omentum at its upper part, the lower part of the membrane adhering to the abdominal wall. The transverse colon was of a long U-shape reaching to the pelvis. The limbs of the U ran parallel to the ascending and descending colon. The hepatic and splenic flexures were normal. Ihseche ('04) saw in a

case a four centimeter opening in the transverse mesocolon through which most of the small intestine reached the lesser peritoneal sac. Some of the coils returned to the greater sac through an opening in the gastro-hepatic omentum and were in relation with the lesser curvature of the stomach. Chalmers ('05) reported a case as a "duodenal hernia through the recessus intermesocolica." He describes an opening in the transverse mesocolon bounded by blood vessels, and measuring about five centimeters in diameter. Two coils of small intestine passed through it into the lesser sac. Perman ('96) records a case having an opening four fingers wide in the transverse mesocolon to the right of the middle line of the body. Through this opening, all of the jejunum and most of the ileum passed into the bursa omentalis. A large opening in the lesser omentum permitted coils to reenter the greater peritoneal sac. Enderlin and Gasser ('06) report cases of intestine lying in the lesser sac. These were most likely far advanced types of "herniae mesocolic media." Mayo ('09) describes a case in which all of the movable small intestine, except the first three and the last twelve inches entered the bursa omentalis through the mesocolic omentum, and escaped through an opening in the gastro-hepatic omentum. In a second case he writes of an opening in the transverse mesocolon in front of the ligament of Treitz, measuring four inches. Five feet of jejunum passed through this opening, carrying with it a layer of the peritoneum derived from transverse mesocolon and bulging out the gastro-hepatic omentum over the lesser curvature of the stomach. This last described hernia having other coverings than the walls of the bursa should probably not be classed as a true bursa omentalis hernia, but rather with those mentioned of Enderlin and Gasser. Prutz ('09) observed a case in which all of the movable small intestine except a terminal twenty inches of ileum had passed through an opening in the transverse mesocolon into the bursa omentalis and then out through an opening in the lesser omentum to hang in front of the stomach. Schumacher ('09) reported finding in a patient an opening measuring five long by one and a half centimeters broad in the transverse mesocolon by way of which the jejunum entered the bursa omentalis and lay between the layers of the great omentum. Stoltzenberg ('10) saw in a case an opening measuring five inches wide in the transverse mesocolon through which the ileum had passed into the bursa. Many of the coils returned to the greater sac by an opening in the lesser omentum. The transverse colon was of a long U-shape, and reached the pelvis as in Schwalbe's case.

Under the variety of bursal herniae that enter by way of an opening in the walls of the great omentum, Baugrand ('60) reports a case with an opening in the great omentum through which the ileum had passed into its interior. Wandel ('03) had a patient with volvulus. At autopsy, he found a common mesentery and an opening in the front wall of the great omentum through which coils of the small intestine entered a cavity between the layers of the great omentum. Hilgreiner ('03) presents the case of a similar hernia, except that the opening was in the lower part of the posterior wall of the great omentum.

The relative frequency in which openings in the gastrohepatic omentum give exit to herniae which have entered the bursa omentalis by means of openings in the transverse mesocolon makes one using such an opening to enter the bursa of special interest. Berg ('97) records such a case. He found a large opening in the gastro-hepatic omentum through which a meter of small intestine and a coil of the colon had entered the bursa.

A number of other cases were found in the literature consulted by the writer which were so indefinite in anatomic data that they are omitted from this brief review.

DESCRIPTION OF THE AUTHOR'S CASE

The subject was a well developed male; the record given to us gave his age as fifty-seven; the cause of death as rheumatism. Besides the condition about to be described, very interesting developmental defects were found in the vascular and urogenital systems. The heart received a right as well as a left superior vena cava, and the kidneys possessed double hili and ureters.

Upon opening the abdomen, part of the colon was observed lying anterior to the exposed part of the liver and stomach. On tracing this part of the colon beneath the left costal margin, it was determined that the gut was folded on itself at the oesophageal end of the stomach and then descended in front of the stomach and right portion of a very large great omentum. This descending part was thus parallel to the ascending colon. When this descending loop reached the level of the fourth lumbar vertebra it was folded under until it reached the right margin of the great omentum beneath which it disappeared.

Upon raising the great omentum to follow the colon it was discovered that all of the small intestine except the duodenum and the terminal ten centimeters of the ileum were within the great omentum, which thus formed a hernial sac. The intestine had entered this sac by an opening in the transverse mesocolon corresponding to the ventral surfaces of the third and fourth lumbar vertebrae. The dorsal border of the opening was found to be a thickened band, running parallel to which was an anastomosing branch between the middle and left colic arteries. On the anterior surface of the great omentum were relatively large arteries derived from the right and left gastro-epiploics. The colic

attachment of the great omentum was limited to the left portion of the transverse colon, as seems to be the rule in cases with very long transverse colons.

Returning to the tracing of this last structure, it was found to cross the vertebral column behind the omental sac and ascend parallel to the descending colon to a normal splenic flexure. Thus the transverse colon was of the U-shape described in the cases of Schwalbe, Stoltzenberg, and others. The caecum, ascending, and all of the transverse colon except the last described ascending limb were very much dilated and devoid of saculation. The colon measured one and a half meters, one-half of the length being in the transverse colon. The small intestine was normal except in relations. The stomach was small and concealed by the colon as previously described, except for a small area of the left side.

A long V- or U-shape transverse colon is not very rare. Huntington states that the relatively large fetal liver gives an oblique direction to the developing transverse colon. With the latter's continued growth, it may form an arch with the summit in the pelvis. This long arch usually disappears with the relative decrease in size of the liver in the later stage of development. Where it persists in the adult it usually, as in the writer's case, forms a large part of the extra length of the large intestine found in these individuals. Transverse colons of this shape are normal in the baboon.

In the reported cases of bursal herniae passing through openings in the transverse mesocolon in relatively few cases was a V- or U-shape transverse colon noted, so the author doubts any causal relationship.

From a study of recorded cases, it seems clear that more than one factor is responsible for a hernia of the bursa omentalis. Moynihan speaks of the frequency of four abnormalities noticed in these cases, viz., the presence of a common mesentery; the absence of fusion of the ascending mesocolon to the primary peritoneum of the posterior abdominal wall; unusually large epiploic foramen, and a very long mesentery. Unfortunately, so few of the reporters have given sufficient anatomical data

in their papers to suffice for statistics, that the writer finds himself unable to state that these conditions are frequent. None of the four abnormalities were present in the case under consideration.

The majority of the observers assign as the reason for the existence of the openings in the transverse mesocolon and omenta an atrophic process involving the area of the future opening and, this atrophy occurring so frequently in essentially the same places has led certain of these observers to postulate also that a slight embryonic defect in these membranes must mark the site for this atrophy.

Stoltzenberg suggests that such a defect in the transverse mesocolon would result from the rotation of the colon. He reports having found in a four month fetus a small opening in the transverse mesocolon against which lay the two highest loops of the jejunum. Also that there was observed a relatively thin area in this location in the membranes of all fetuses and young infants he had been able to investigate at post mortems. Schwalbe also writes of having observed such depressions. The possibility has suggested itself to the author that such depressions might be explained by assuming that the arteries as they pass ventral in the membrane to reach the viscera cause slight ridges to appear in the mesenteries, the areas between appearing as depressions. The space between the anastomotic branches of the middle and left colic arteries might thus become such a slightly depressed area in the peritoneum. A number of observers have noted that these two arteries bound the opening found in the transverse mesocolon in these cases.

Stoltzenberg has pointed out that this area between these vessels may perhaps present a region in which the abdominal pressure would find a path of least resistance to what he terms the "stomach-liver niche," located above the transverse colon. He fully appreciates that this pressure would be great in the relatively small abdominal capacity for the rapidly developing coils of the intestine as found in a fetus.

On the other hand, these bursal herniae have so frequently returned to the greater peritoneal sac through an opening in

the gastro-hepatic omentum not bounded by vessels; in Berg's case, the hernia entered the lesser sac at this place, and in Blandin's case, in which the hernia, after having entered by way of the epilotic foramen, emerged by an opening in the transverse mesocolon; that the writer thinks this explanation of fetal pressure in an upward direction on vessel bounded depressions is inadequate. Postnatal pressure as a cause has perhaps been more frequent than prenatal. The clinical data in these cases show the frequency of ulcer, cancer, and hour-glass stomach in these subjects; conditions in which one would have violent vomiting and peristalsis to increase abdominal pressure.

The fact that in only a few cases have these hernias occupied the cavity of the great omentum which exists during the first and often the second year of life seems to the writer a strong point in favor of postnatal origin. Why may not gravity bring the intestinal coils of such a hernia down into this space instead of their remaining in the retrogastric portion of the bursa omentalis as is true in most of these cases? The presence of long and common mesenteries noted in a number of these cases would make this possible. Again, this form of hernia has not been observed in any subject in the first few years of life. The vast majority have been adults.

It seems plausible that in the cases where the hernias were found in a cavity of the great omentum these individuals possessed a cavity long after the normal time of fusion of the lamellae of the great omentum. When pressure atrophy effected an opening into the bursa omentalis of such subjects coils of the intestine would gravitate into the lowest part of that cavity.

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THE DEVELOPMENT OF ERYTHROCYTES FROM HEMOGLOBIN-FREE CELLS AND THE DIFFERENTIATION OF HEART MUSCLE FIBERS IN TISSUE CULTIVATED IN PLASMA

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TWO FIGURES f

The results of the small amount of hematological investigation which has been done by the use of tissue cultures shows the enormous possibilities of this method when applied to the investigation of problems for the solution of which the examination of smears and the section technique have proved inadequate.

Among a series of cultures made last winter were some for which the explanted tissue was taken from the area opaca of chick embryos—before the formation of blood islands and the elaboration of hemoglobin. All of these cultures were planted in the blood plasma of an adult hen after Harrison's method, and in some of them, hemoglobin-bearing cells developed from amoeboid colorless elements apparently of mesoblastic origin.

In all of the cultures (one hundred and twenty) there was vigorous cell proliferation and migration which began within an hour after the culture slides were placed in the incubator and continued for from three to four days. This growth was so exuberant and cell migration was so rapid that in twenty-four hours the layer of new cells was perceptible without the aid of a lens, and in forty-eight hours a ring of new tissue three or four mm. in diameter was formed about the original explant. In some cases long cords of cells grew off from the edge of this ring for a distance of two or three mm., and the cells at the ends of the cords spread out on the cover glass to form a secondary growth or colony connected to the original transplant and its surrounding ring of new tissue by a thick strand of cells.

It was probably this over-luxuriant cell growth with the resulting proportionate increase in the bulk of the culture and the accumulation of the waste products of the metabolism of the new and rapidly growing cells, which caused the appearance of signs of degeneration and the cessation of growth in such a relatively short time. Cultures of ordinary embryonic mesoderm in a blood plasma medium will grow without signs of degeneration for seven or eight days without subculturing or plasma renewal but the initial signs of growth and migration rarely appear before the culture is twenty-four hours old. Throughout the life of the transplant, growth is much slower in cultures of mesoderm than in these cultures from the area opaca of very young embryos.

Microscopic examination revealed the presence of mesenchymal cells in the new growth, but by far the greatest bulk of culture grown tissue was composed of cells which were evidently descended from the endoderm of the yolk sac wall. These (fig. 1) were huge cells spread out in a rapidly broadening syncytium-like membrane in which there were no visible boundary lines between the cells. Easily distinguishable from the mesodermal cells by their size, their huge nuclei, and the lacy appearance of their cytoplasm, these endodermal elements may be seen to be full of fat and yolk granules. In fixed preparations stained with Giemsa's stain, a fine open-meshed spongioblastin network was apparent, the threads of which had a granular appearance. The huge nuclei, homogeneous in the fresh cell, finely granular in preparations fixed in osmic acid vapor, were marked by the presence of two or three darkly stained chromatic masses or basophil nucleoli.

The endodermal cells in fresh preparations had, in common with all other cells grown in plasma, a clear homogeneous ectoplasm at their periphery surrounding a granular endoplasm in which the paraplasmic structures and cytoplasmic granulations were contained.

Scattered about among these endodermal cells and in the clear area of plasma about the transplant, young erythrocytes developed in many cultures from undifferentiated, highly amoeboid,

very small mononuclear elements, with a strong basophilic, sometimes vacuolated cytoplasm, and a small round nucleus with a coarse chromatin network (fig. 2a). The process of erythrogenesis was initiated by a cessation of amoeboid movement.

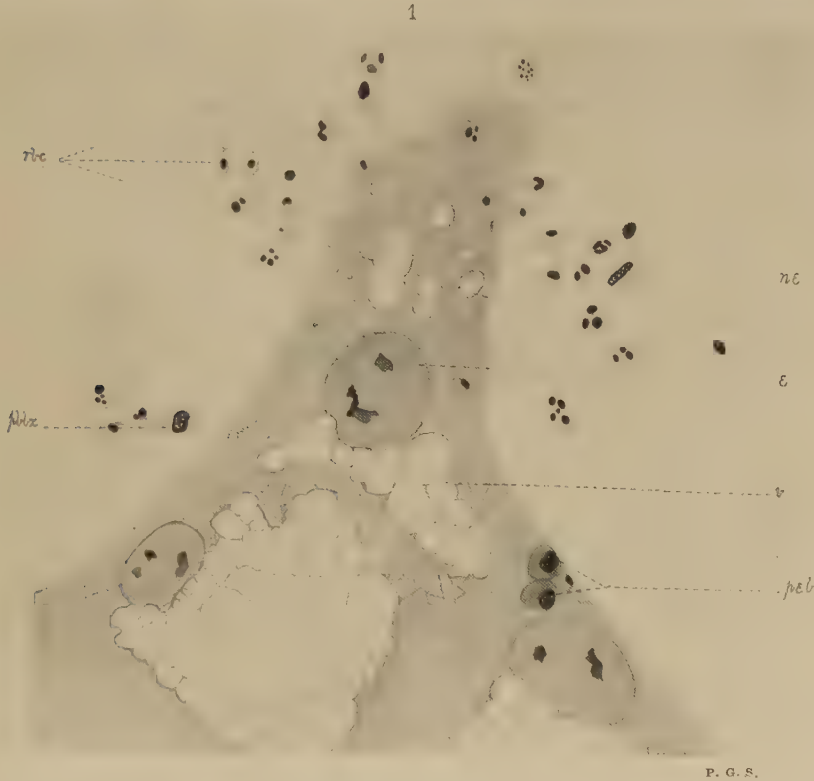


Fig. 1 Edge of a 48 hour culture from the area opaca of an 18 hour chick. Fixed in absolute methyl alcohol. Stained with Giemsa. Zeiss drawing prism. Kompens ocular No. 8 and 4 mm. apochromatic objective. Three large cells from the yolk sac wall and a large number of culture grown erythrocytes. *e*, nuclei of the endodermal cells; *pblz*, primitive blood cells; *pbl*, primitive erythroblasts; *rbc*, abnormal forms of young erythrocytes; *nec*, normal chick red blood cell.

The cell withdrew its processes and became round, the cytoplasm, however, still retained its affinity for basic stains (fig. 2b). The round hemoglobin-free cells still had the power of multiplying by mitosis, but usually hemoglobin was elaborated in

their cytoplasm without further division, with the resulting formation of the hemoglobin-bearing erythrocytes.

In this way a small number of normal red cells were produced in cultures, but most of the erythrocytes differed in many respects from the red blood cell as one finds it in the vessels of the embryo when it has developed under normal conditions in the



P. G. S.

Fig. 2 Young forms of blood cells from the above preparation. Zeiss drawing prism. Kompens ocular No. 8 and 2 mm. apochromatic oil immersion objective. *a*, primitive blood cells. No. 1 is dividing by mitosis and has a vacuolated cytoplasm. *b*, Primitive erythroblasts. The nuclei still have a definite chromatin network and the cytoplasm is polychromatiphilic. No. 6 is dividing mitotically. *c*, abnormal red blood cells with pyknotic nuclei.

blood islands or vessels, connective tissue or the embryonic liver, bone marrow or spleen (fig. 2c). In the first place, the erythrocytes were very much smaller than the normal corpuscle of the chick embryo, the average diameter being about 4μ . Again, they had not the oval shape which characterizes the red blood cell of the chick. Most of them were spherical, or round discs. Poikilocytes were common and the cytoplasm of some

was perforated by vacuole-like apertures. A few had polychromatophil cytoplasm but none showed any evidence of stippling.

Not less peculiar were the results of changes which took place in the nucleus of the primitive blood cell and the young erythrocyte. In none of these small red blood cells does one see the characteristic long, oval nucleus, with its rounded ends, but the nucleus is represented by one or more small homogeneous deeply stained fragments or rests (fig. 2c). The chromatin of the original functioning nucleus seemed to be concentrated into masses within the nuclear area, the breaking down of which released them to scatter about through the cell cytoplasm. A cell may contain six or seven of these small chromatic masses, but usually two or three are found in one cell.

The morphology of the abnormal young forms is remarkably like that of young mammalian red blood cells with pyknotic nuclei and 'Jollykörper' or homogeneous chromatin rests, but the physiological significance of this aberration from the normal is uncertain. The dwarfing of the blood elements has its beginning in the primitive blood cells which are very much below the size of the corresponding element when developed under normal conditions, but the agents which produce this dwarfing we can only guess at.

The appearance of undersized, atypical forms among red blood cells developed under cultural conditions is not surprising. Indeed it is not to be expected that the development and differentiation of the highly specialized cells of warm blooded vertebrates should proceed unaltered when cells as low in the scale as the protozoa exhibit pronounced deviations from the normal when removed from their accustomed environment and forced to grow on media to which they are unused.

These erythrocytes have come into being in a medium clogged with the waste products of the metabolism of a large number of rapidly growing cells, and furthermore, were, in all probability, insufficiently supplied with oxygen, since oxygen penetrates these clots of plasma with difficulty. Both of these conditions may have contributed toward the production of atypical dwarf

forms, but it will take further and careful study to determine which factor was chiefly responsible.

In connection with the genesis of dwarfed, abnormal red corpuscles in culture, it is interesting to note certain peculiar, probably degenerative changes in the form of normal chick red blood cells which have remained in vitro for some days in plasma clots. They are commonest in cultures from the embryonic spleen after three or four days incubation, and they consist of the drawing out of the poles of the affected cell into one or more long processes. One or both poles of the erythrocyte may be affected. These long, flagella-like projections appear, when examined in a fresh condition, to be beaded for all or part of their length and to terminate in a small round knob. They are actively motile, whipping back and forth at the end in a manner suggestive of the movement of cilia. They are similar to, but probably not identical with, the flagella which Kite has described as occurring under special conditions about the periphery of the mammalian erythrocyte.

In some of these cultures, parts of the embryonic body were included in the transplants, and in these an interesting phenomenon was observable. After an incubation period which varied from twenty-four to thirty hours, the heart muscle developed, assumed its function, and began to beat rhythmically. Regularity in beat was not maintained for over twenty-four hours, after which time the rate became slower and slower until it ceased. Even after cessation, however the pulsation could, for a time, be reinduced by mechanical or thermal stimulation. Especially were the heart muscles sensitive to cold. Removal from the incubator to the cold stage of the microscope was answered by renewed and vigorous beating long after the embryonic cardiac muscle had ceased to respond to other stimuli. Burrows and others have shown often that heart muscle cells will contract rhythmically when isolated from contact with others of their kind, but these cultures show that the embryonic cell which is destined to become heart muscle will differentiate and begin to function even though removed from its normal environment before differentiation has begun.

While the blood corpuscles developed in these cultures of the primitive area vasculosa were for the most part abnormal in size and shape, at least a study of this series of cultures indicates that development and differentiation of hemoglobin-bearing cells is possible in tissue transplants removed from the normal environment to a culture medium, and shows that with an improved technique we may be able to approximate a norm closely enough to solve by this method many of the problems of erythro- and leukocyto-genesis. Also the development of erythrocytes from amoeboid colorless ancestors and the specialization and assumption of rhythmic contractility by embryonic mesoderm cells under cultural conditions, together with Harrison's demonstration of the outgrowth of the nerve fiber, show that the life of cells in culture is not merely a series of 'survival phenomena' on a downgrade of progressive differentiation.

THE USE OF EARLY DEVELOPMENTAL STAGES IN THE MOUSE FOR CLASS WORK IN EMBRYOLOGY

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The primary object of the course in embryology offered in medical schools is undoubtedly to give the student an idea of the principal features of human development. Human material, however, is available for embryological study to only a limited degree. Consequently the instructor must look to other mammals and frequently to the lower vertebrates, or even invertebrates, for illustrative material. This means that a course in 'human embryology' must of necessity be to some extent a course in comparative embryology, but one in which, owing to the usual briefness of the course, each lower form is slighted as much as possible in order to leave time for considering higher forms. Such a procedure robs the course of much of its value as a comparative study and, moreover, is in danger of leaving the student with a confused rather than a clarified conception of ontogeny in general. Under these conditions it seems desirable to select for the routine course in embryology the fewest number of forms that will illustrate all the essential stages in mammalian development.

The problem of selecting these few forms for study is by no means an easy one. The value of the pig for the study of organogeny is generally recognized. Suitable pig material, too, may sometimes be had for the study of the somites and germ layers, but in general the chick has to serve for this part of the course. For the study of germ cells, fertilization and cleavage, various forms, chiefly invertebrates, are used. The purpose of this paper is to call attention to the availability, and suitability, of mouse material for the study of all steps up to the end of the blastocyst stage.

The earliest stages in a considerable number of mammals have been studied in detail and there has been found to be a fair degree of resemblance between the different forms. For medical embryology, and for the more extended courses in comparative embryology, there can be no question as to the desirability of showing the student these mammalian ova sectioned in situ within the ovary and uterine tube. The chief obstacle to their use no doubt has been their inaccessibility. In the case of the mouse, however, there is a considerable literature¹

¹ A good bibliography will be found in G. Carl Huber's paper on "The development of the Albino rat, *Mus norvegicus albinus*. I. From the pronuclear stage to the stage of mesoderm anlage; End of the first to end of the ninth day." *Memoir of the Wistar Institute of Anatomy and Biology* No. 5, 1915.

relating to the early stages and we have found that suitable material for class work may be obtained with little difficulty.

We first made the experiment of using early stages of the mouse for class study in 1912, with results so satisfactory that the material has been used with the three succeeding classes. With the hope that our experience may be of some value to others, the following brief account of the method of handling the mice and the average cost of the sections is presented. This discussion may be prefaced by a description of the early part of our embryology course as it is being given at the present time.

At the beginning of the course male and female mice are dissected sufficiently to show the form and relations of the genital glands and ducts. Living sperm cells are then studied in salt solution. The ovary is dissected and examined under hand lens or binocular microscope. Next spermatogenesis is studied in sections of the testis. In sections of the ovary the student finds and draws a series of stages showing the growth of the ovum and development of the follicle. The last stage in this series shows a follicle in which the corona radiata cells have assumed the appearance seen at ovulation and surround an ovum which has given off the first polar body and formed the second maturation spindle. From the uterine tube and uterus sections are studied and drawings made of the following stages: pronuclei and second polar body, two and four cell ova, late cleavage, morulae and stages in the formation of the blastocyst. Beyond this stage mouse embryos are not so satisfactory owing to the elongation of the blastodermic vesicle and the excessive development of entoderm. Some of the later stages of implantation on the other hand are very instructive.

The prime essential for securing this material is a stock of vigorous mice of good breeding age, the best season for the work being in the spring from February to May. In our experience young females from three to four months old have proved most satisfactory. While older females occasionally breed regularly they are much less dependable. Detailed accounts of the methods employed in securing the material will be found in several of the papers referred to in the footnotes, but it may be well to summarize briefly a few points in the breeding habits of the mouse and to indicate what seem to be the best methods of taking advantage of them.

Female mice of proper age tend to ovulate approximately every twenty-one days during the spring months. They almost invariably ovulate within a few hours after parturition. This latter fact is important since it enables one to know the approximate time of an ovulation without the necessity of keeping any special records. We have found it most convenient to keep the mice in small groups consisting of one or two males and six or eight females. They may be fed on dog biscuits, cracked corn and oats, with a little salt and occasionally a few sunflower seeds. Whenever a female is seen to be pregnant she is isolated, and kept till parturition occurs. Suckling the young may prolong the duration of the next pregnancy by as much as 50 per cent

but it has not been shown definitely that it has any effect on the progress of development during the first four days in which period most of the desired stages are to be found. Nevertheless, since the young must be sacrificed anyway, we have made it a practice to kill them at once.

When young mice are expected we have found it best to look in the cages early in the morning and again just before leaving at night. Young are more frequently found in the morning, but a considerable number are born during the day. Not infrequently a female will be found in the process of parturition. Mark and Long find that the first polar body is formed and ovulation occurs from $13\frac{3}{4}$ to $28\frac{1}{2}$ hours after parturition. In getting these stages for the class it is best to select females that are found with young at the night inspection. These need not be mated and should be killed during the following forenoon. It will probably be found advantageous to leave these stages till the last since some of the ova expected to show fertilization will be found in reality to be in earlier phases of maturation.

For all subsequent stages insemination is necessary. Artificial insemination was practiced by Mark and Long² but for the purposes under consideration it has few advantages. The method that has proved most satisfactory in this laboratory is as follows. A few mature males are isolated and kept in separate cages. When it is desired to mate a female, she is put in the cage with one of these males. It is essential that the male be left in his home cage, otherwise he will lose much time in exploring the new surroundings. This is not true of the female. If the female is in the proper condition successful coitus usually occurs within 20 minutes. Coitus has been described in detail by Sobotta.³ Following the sperm the contents of the seminal vesicles are discharged into the vagina forming a large, white plug which may be easily detected for several hours. Taking advantage of this fact, the female may be left in the cage for an hour or more at the end of which time it can be definitely determined whether or not coitus has occurred. If the female will not mate within an hour she is removed and given another trial from six to twelve hours later. During the active breeding season only a few females refuse to mate. This procedure gives a much higher percentage of successful seminations than is reported for the artificial method and, unless extreme accuracy is required, takes less time.

The best time for insemination is probably about twenty-four hours after parturition. Five or six hours after the insemination ova in the tube will usually be found to contain two pronuclei. This is one of the easiest stages to obtain. Specimens taken at twelve hour intervals for the next four days give a fairly complete series of stages up to the formation of the blastocyst.

² Long, J. A. and Mark, E. L. "The maturation of the egg of the mouse." Carnegie Institution of Washington Publication No. 149, 1911.

³ Sobotta, J. "Die Befruchtung und Furchung des Eies der Maus." Arch. f. Mikr. Anat., Bd. 45, pp. 15-93, 1895.

It will be seen from the foregoing account that the securing of these stages requires no unusual skill or special apparatus. All of this work may easily be done by an intelligent technician. The method outlined here has an advantage in that it results in a greater variety of stages and a reduced expenditure of time. With the exception of an occasional individual in which for some reason ovulation or semination have failed to occur every mouse killed yields some interesting stage. Both sides show ova in the same state of development, so that two sets may be obtained from each mouse.

The mice are killed either by chloroform or by decapitation. The ovaries and uterine tubes are then fixed in ordinary Zenker's fluid or the Mark and Long modification and subsequently imbedded in the usual manner. It is found desirable to cut the sections 8 micra thick. An ovary and uterine tube yield sections enough for two 36 x 75 mm. slides. It would be an easy matter to separate the ovary and tube, in which case either one would go on a single slide. Kirkham⁴ gives a method for locating the position of the ova before the slides are finished. By this means small slides could be used and occasionally several specimens could be made from one tube. Alum haematoxylin and eosin give a satisfactory stain.

It is in the small size of the parts to be sectioned, the possibility of getting any desired stage, and the ease with which the adults can be handled that the chief value of the mouse as a source of embryological material lies. The current price of adult mice is from 20 to 25 cents each and we find that it costs about 4 cents a month per mouse for maintenance. Including the cost of males and unproductive females our experience indicates that the average total cost of each usable ovary and tube, properly fixed, stained and mounted ready for study is 54 cents.⁵ This figure includes the cost of all reagents, slides, covers, etc., but does not take account of the time required. This is perhaps rather more expensive than would be the corresponding stages of an echinoderm, for instance, but it is so much more valuable that the additional expense seems justifiable. It might be mentioned incidentally, that in our laboratory course one specimen of each stage for every five or six students has proved adequate. At this rate, provided ten stages are used, the initial cost will be seen to be about \$1 per student. Except for breakage, of course, the slides may be used indefinitely.

⁴ Kirkham, W. B. "The maturation of the mouse egg." Biol. Bull., vol. 12, no. 4, pp. 259-265, 1907.

⁵ This statement is based on figures obtained in connection with the preparation of a series of mouse embryos and ova collected in the spring of 1915, at which time all the costs in any way incident to the work were tabulated as accurately as possible. The data are available to anyone who may wish to repeat the work.

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

THE RAT. Reference tables and data for the albino rat (*Mus norvegicus albinus*) and the Norway rat (*Mus norvegicus*); Compiled and edited by Henry H. Donaldson; Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1915.

This is primarily a reference work for those interested in the rat as a laboratory animal. It is based chiefly upon the extensive published investigations of Professor Donaldson and his colleagues, but includes some work hitherto unpublished, together with available data compiled from various sources. The introduction includes a discussion of the classification of the common rats, and a history of their migrations. The book proper is divided into two parts, the first dealing with the common albino rat, the second with the wild Norway rat (from which the albino has been derived). Various chapters deal briefly with the biological characters, heredity, anatomy (including histology and embryology), physiology and pathology. A convenient index is provided.

The main purpose of the work is to present those data which can be systematically arranged in quantitative form. The data are arranged chiefly in tables and graphs showing the growth in size and weight of the whole body and of the various constituent parts, systems and organs. Tables of growth in the chemical constituents are also included. The tables are conveniently arranged for reference, so that for a given animal the normal weight of any corresponding organ is immediately obtained.

While these normal tables by no means eliminate the necessity of adequate controls in experiments upon the rat, they furnish a very useful and convenient basis whereby the deviations found in the various local strains used can be compared and measured. If the experimenters will utilize the tables in the manner recommended, it will make possible a standardization of their results upon a basis of comparison much better than any hitherto available.

The necessity for more complete data upon the normal variability of various biological characteristics is especially evident when we consider that without this knowledge it is impossible to draw trustworthy conclusions from the apparent results of experiments, or to judge as to whether the number of controls used is probably adequate. The apparent discrepancy in the variability of the body weight of the albino rat as found by Jackson (table 58) and by King (table 67) is probably due to the fact that the former is based upon a 'random sample' of the general population, whereas the latter is based upon a study of selected litters of strong and vigorous individuals (*Anat. Rec.*, vol. 9, p. 751), among which less variability might be expected. The great advantage of controls from the same litter is emphasized by evidence that fraternal (intra-litter) variability is but little more than half that of the general population.

For data not included in the scope of the present work (which deals with the normal rat only), references are grouped at the ends of the various chapters. A general bibliography of 948 titles of works dealing wholly or partly with the rat is also given. Even this large list is incomplete, since numerous bacteriological and descriptive zoological references were intentionally omitted.

The already large number of investigations based upon the rat is likely to increase greatly in the future, as the advantages of this animal for experimental work become better known. Such work will be greatly facilitated by Donaldson's manual. It is to be hoped that the more evident gaps in our knowledge of the rat, especially in its histology and embryology, will be speedily filled; so that for at least one laboratory type biometric norms throughout the life cycle will be available. An albino rat colony will thus perhaps in the near future become a recognized necessity in every well-regulated biological laboratory.

C. M. JACKSON.

AMERICAN ILLUSTRATED MEDICAL DICTIONARY. A new and complete dictionary of terms used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, Veterinary Science, Nursing, Biology, and kindred branches; with new and elaborate tables. Originally published in 1900. Eighth Revised Edition. Edited by W. A. Newman Dorland, M.D. Large octavo of 1135 pages, with 331 illustrations, 119 in colors. Containing over 1,500 more terms than the previous edition. Flexible Leather, \$4.50 net; thumb index, \$5.00 net. Philadelphia and London; W. B. Saunders Company, 1915.

From the Preface. The aim of the author of this work has been to produce, in a volume of convenient size, an up-to-date medical dictionary, sufficiently full for the varied requirements of all classes of medical men. Physicians and students have long felt the need of such a work. The book does not claim to be an encyclopaedia; is a dictionary, a concise and convenient word-book, aiming to furnish full definitions of the terms of medicine and kindred branches, and such collateral information as medical men generally would be likely to look for. The author has sought a middle course between the large, unwieldy lexicon and the abridged students' dictionary, avoiding the disadvantages of each.

The present edition has been carefully revised throughout, so thoroughly, in fact, that it was necessary to make entirely new plates for it. Several hundred new terms have been defined, and the text matter has been increased by 30 pages.

During the past two years a multitude of new tests, both clinical and laboratory, have been published, with the result that the list of tests in the Dictionary has been greatly increased. This list has grown from ten pages in the original edition to nineteen pages in the present one. This is only a single instance of the constant and rapid increase in the terminology of medical science.